Long-Term Variability of Plankton Phenology in a Coastal Mediterranean Time Series (LTER-MC)

Thesis

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Long-term variability of plankton phenology in a coastal, Mediterranean time series (LTER-MC)

Thesis submitted in partial fulfillment of the requirements

for the degree of Master of Philosophy (MPhil) in Science

September 30, 2013

by

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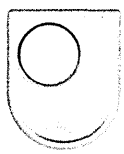

DATE OF SUBMISSION: 30 SEPTEMBER 2013
DATE OF AWARD: 6 DECEMBER 2013

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P.O. Box 1346
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ABSTRACT

Long-term patterns of phenology were identified for 104 phytoplankton and 88 zooplankton taxa at a coastal, Mediterranean time-series station (LTER-MC) in a 23 year period (1984-2010). 25 phytoplankton taxa and 41 zooplankton taxa showed a significant long-term change in at least one phenological event, including only a few taxa among the numerically dominant ones. The most frequent changes in phytoplankton taxa (28%) were recorded in the onset of phenology, while most zooplankton (30%) taxa changed the month of seasonal peak. The changes in phenology recorded in phytoplankton were significantly correlated with salinity anomalies for 10 taxa and with temperature anomalies for 8 taxa. Among zooplankton, 12 taxa had changes in phenology that were significantly correlated with chl *a* anomalies and 9 taxa with temperature anomalies. An overall comparison of changes in plankton taxa belonging to 7 functional groups revealed a long-term earlier trend between the end (2010) and the start (1984) of the time series. The strong trend was driven by a significantly earlier trend in the second part of the time series (1995-2010). The observed changes in phenology and their relationships with anomalies of the environmental parameters indicate that most variability and changes occurred in spring and in autumn. Very few taxa changed phenology in summer.

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1.1 Phenology in aquatic and terrestrial ecosystems

The timing of natural events and their study in relation to the environment and climate change is the interdisciplinary subject of phenology. Phenological studies encompass terrestrial and aquatic ecosystems and range in scale from remote sensing measurements of land-cover "greening" events using NDVI (Pettorelli *et al.*, 2005), to satellite ocean chlorophyll *a* concentrations (D'Ortenzio *et al.*, 2012, Lavigne *et al.*, 2013), and at the organismal level - insect-pollinator studies (Ellwood *et al.*, 2012, Singer & McBride, 2012), and freshwater and marine plankton (Caron & Hutchins, 2013, Edwards & Richardson, 2004, Ji *et al.*, 2010, Mackas & Beaugrand, 2010, Mackas *et al.*, 2012, Thackeray, 2012) to highlight just a few.

In terrestrial systems, observations of species occurrence, abundance and distribution patterns have revealed a coherent response in phenological events associated with impacts of climatic change such as an accelerated timing of flowering with an earlier climatological spring (Cleland *et al.*, 2007, Ellwood *et al.*, 2012, Parmesan & Yohe, 2003) a mismatch between flowering and insect pollinators resulting from a change in flowering occurrence (Kudo & Ida, 2013), shifts in the distribution patterns of migratory butterfly species and birds in response to regional warming (Parmesan *et al.*, 1999, Thomas & Lennon, 1999), responses to winter and spring warming in earlier onset of flowering temperate plants (Cook *et al.*, 2012), and earlier leaf-out events in trees (Polgar & Primack, 2011). These changes in phenology have been observed over spatial and temporal scales (Stenseth & Mysterud, 2002), and at species and population to community levels (Cleland *et al.*, 2012, Ovaskainen *et al.*, 2013).

Phenological observations in aquatic systems have elucidated the biological response to changing climatic conditions in freshwater lake systems such as altered spring bloom phenology as a response to nutrients, and temperature (Feuchtmayr *et al.*, 2012), and shifts in seasonal blooms in response to changing conditions at high latitudes (Kahru *et al.*, 2011), and in marine systems, a similar response in altered phenology has been

observed in biological communities as a result of rising sea surface temperature (Aberle *et al.*, 2012, Berger *et al.*, 2007, Genner *et al.*, 2009, Olita *et al.*, 2007, Vargas-Yáñez *et al.*, 2008), changing salinity due to increased precipitation events (Ji *et al.*, 2007, Trenberth, 2011), and trophic level responses among phytoplankton and zooplankton that have been the subject of many recent efforts to disentangle the impact of global climate change (Menzel, 2002).

1.1 How do we measure phenology?

Phenology can be measured at temporal and spatial scales using detailed observations of timing in plant and animal communities along with measurements of corresponding environmental conditions and geographical location. Phenological measurements include describing and quantifying the occurrence of a wide range of phenological events that are specific for terrestrial or aquatic system. These "phenological events" consist of quantitative and qualitative observations of biological processes such as the date of occurrence of bud-burst in terrestrial plants, physical changes in the ecosystem such as ice-out in lakes, and spring blooms of phytoplankton in coastal and oceanic systems. A review of quantitative methods used to identify phenology in marine plankton by (Ji *et al.*, 2010) highlighted the need for a standardized set of interdisciplinary methods outlined by (Hudson, 2010). Phenology can be used as a tool to understand and predict the response of biological systems to future climate scenarios; however this emerging science requires integrating different disciplines to acquire combined knowledge of the impacts (Visser & Both, 2005).

1.2 Long-term observations of ecological change

In recent years, analysis of long-term records has revealed evidence of climate change in ecosystems (Burrows *et al.*, 2011). Long term data sets provide an approach to address patterns of change in natural phenomena (Magnuson, 1990). Furthermore, comparative time series analysis provides a greater understanding of climatic responses at regional and global scales (Mackas & Beaugrand, 2010).

Several long-term phenological observation programs maintain ongoing records of terrestrial events eg. Nature's Calendar in the UK, and the USA National Phenology Network in North America. The emerging field of data science or "ecoinformatics" has responded to the influx of phenological observations and analysis needs with a range of projects such as PANGEA, The Knowledge Network for Biodiversity-KNB, and others such as the National Ecological Observatory Network-NEON and the international Long Term Ecological Research (i-LTER) to support the collection, storage and use of long-term ecological data. Additional efforts to digitize more than 480 long-term time series in central Europe have resulted in the public **P**lant-**P**henological **O**nline **D**atabase (PPODB) which is a collection of over 16 million plant-phenological observations from about 9000 stations covering 130 years (1880-2009) (Dierenbach *et al.*, 2013).

In addition to long term ecological time series and monitoring efforts, public data collection efforts termed "Citizen Science" have emerged as a valuable tool to assist scientists in the collection of phenological data. Citizen science relies on the volunteer efforts of public citizens to collect observations (often qualitative) records of natural events. Despite the possible limitations to using citizen science and scrutiny of data quality (Lawrence, 2009), citizen science efforts have provided insight into phenology. In recent years, historical hand written observations (dating as early as 1851) of North American flowering dates, bird arrivals and insect trapping records have been digitized to reconstruct long-term phenological patterns (Primack & Miller-Rushing, 2012). The published

ecological literature and available datasets of phenological observations is vast and presents both a formidable challenge and opportunity for scientists to assemble a coherent understanding of the impacts of climate change on different organisms and ecosystems. With new tools and expanding computing power, the scientific community in turn has responded with an ecological imperative to archive and make publically funded research available in order to facilitate understanding of global change impacts on human and natural ecosystems (Pau *et al.*, 2011).

1.3 Plankton as indicators of climate change

At the base of freshwater and marine aquatic food webs are microscopic plankton consisting of auto and heterotrophic phytoplankton and micro and mesozooplankton. Planktonic organisms play a fundamental role in both freshwater and marine ecosystems by shaping complex food webs and contributing to biogeochemical fluxes. At species and population level, plankton can be characterized by their life history traits and their rapid response to environmental variability (Litchman *et al.*, 2013). Since plankton communities are short-lived and exhibit direct physiological and indirect (eg. phenological) responses to climatic conditions, they are often considered important sentinels or indicators of climate change (Richardson *et al.*, 2012).

Similar to terrestrial systems, aquatic systems fluctuate at seasonal scales and are driven by complex physico-chemical interactions between organisms and environmental mechanisms (eg. light, temperature, dissolved nutrients), and biological interactions among organisms (predator-prey interactions, grazing and production) (Reynolds, 2006). Seasonal variability in the timing and magnitude of biological production by phytoplankton can result in a coupled or uncoupled response by higher trophic level organisms from zooplankton to fish. In aquatic systems, seasonally driven phytoplankton blooms create favorable conditions for predatory aquatic organisms such as *Daphnia* which in turn

exploit the available food resources (Winder & Schindler, 2004). When a shift in the timing of seasonal bloom occurs, a corresponding change in the occurrence (*phenology*) may result in other trophic levels.

1.2 Measuring change in plankton communities

Plankton abundance and composition varies over annual and inter-annual scales and can be used to extract information such as phenology and response to climate change (Winder & Cloern, 2010). In order to quantify plankton phenology, data must be sampled at an adequate resolution to capture seasonal patterns of variability that characterize both taxa and bulk properties of the plankton system (such as biomass). Some phytoplankton species are responsible for recurrent seasonal blooms that result in significant biomass accumulation. This is an important part of the annual patterns in a plankton community. If a bloom persists for only several days, a monthly survey the window of sampling time may not capture this seasonal phenomenon Comparative studies of plankton time series attempt to address patterns of variability by comparing similar species in different geographical regions or similar environmental correlates such as temperature, nutrients, etc. (Mackas *et al.*, 2012).

Changes in seasonal and long term abundance within plankton communities is likely driven not by a single species, but rather by a diverse assemblage comprised of several numerically dominant species within each functional group. Although phenology has been considered for large taxonomic groups; eg. copepods, diatoms, dinoflagellates , chl *a*, this approach may mask the important contribution of key species (Edwards & Richardson, 2004). Conversely, if several species are responsible for the seasonal signature of abundance, phenology analysis that considers combined taxa will show a similar pattern. Therefore, an important consideration in any phenology study is the resolution of biological information. Most often, species level data is less frequently available, and

studies must rely on measured bulk chlorophyll, or even satellite surface chl *a* (D'Ortenzio *et al.*, 2012, D'Ortenzio & Ribera d'Alcala', 2009).

1.2.1 Climate change revealed by phenological patterns

Changing temperature and climate patterns have the potential to alter phenological events and disrupt ecosystem function in new ways not previously observed (Diez *et al.*, 2012), which underscores the importance of understanding the mechanisms of change and the subsequent response (Pau *et al.*, 2011). Climate change is considered an important driver of phenological change in aspects of plankton ecology such as the timing of annual spring phytoplankton bloom, the timing of reproduction in zooplankton, and the physiological response to elevated temperature conditions (Caron & Hutchins, 2013). Therefore, phenology can be applied to understand the biological response of planktonic organisms to global climate change (Ji *et al.*, 2010).

Since plankton maintain a critical role in ecosystem function, continuous records such as the Continuous Plankton Recorder (CPR) survey can be analyzed in parallel with hydro-climatic data to determine the response of pelagic communities to changing environmental conditions across different geographic regions (Beaugrand & Reid, 2003, Pen *et al.*, 2006, Reid *et al.*, 2003). One of the longest continuous records of marine phytoplankton and zooplankton abundance in the world (the Continuous Plankton Recorder survey - CPR) initiated in the 1931 revealed that the timing of spring phytoplankton populations influenced the copepod community in parallel with the length of time a food source is available (Colebrook, 1979). More recently updated CPR records identified a large change in the marine ecosystem (termed a regime shift) that occurred in the North Atlantic (1996-1997) and a corresponding relationship between decreasing salmon stocks and increased temperature (Beaugrand & Reid, 2003).

1.2.2. Phenology as a consequence and driver

Shifts in phenological timing as a result of changing climate can result in a complex cascade of changes in other phenological events which in turn can alter the structure of the natural environment from community to ecosystem level. Changes in the phenology of different populations can result in overall changes to the trophic level coupling when phytoplankton and zooplankton populations do not occur within the same phenological time frame. The timing of interacting species within a community is described as the process of *synchrony* or more generally when the timing of one population has a related dependence on the occurrence of another population. The importance of timing within plankton populations was first elucidated by the fisheries biologist, D.H. Cushing through his research on fish stock recruitment patterns (Cushing, 1989, Cushing *et al.*, 1990). Since phytoplankton constitute a primary food source for marine zooplankton and fish larvae, asynchronous timing of these events could result in a decline or collapse of developing fish populations. Cushing proposed the ecological theory known as the *trophic mis-match hypothesis*. This states that variability in the timing of plankton production leads to variability in larval mortality and consequent year class strength of fish populations.

Since Cushing's early work, phenological studies have aimed to identify responses among interacting trophic levels with mixed success. The mis-match in seasonal timing of an annual spring phytoplankton bloom with corresponding zooplankton populations has been correlated with climatic changes (Edwards & Richardson, 2004). Similarly, changes in zooplankton phenology have been attributed to climatic factors (Greve *et al.*, 2005). In marine planktonic communities, the asynchronous timing of populations may be driven by shifts in phenology as a result of climate change (Edwards & Richardson, 2004, Thackeray, 2012, Thackeray *et al.*, 2010).

Increased global land and sea surface temperatures have been documented as a result of global change (Hansen et al. 2006). Current predictions state a warming of 2-6 °C

in surface ocean waters (Sarmiento *et al.*, 2004). Global changes in surface land, air and water temperatures may exert a direct effect on physiological processes of organisms and could alter spatial distribution patterns and life history events. The effects of climate change can be summarized as either direct or indirect. Direct effects as a result of response to chemico-physical changes in the environment result in altered biogeochemical ratios, changes in primary productivity and species abundance and composition. Indirect effects such as alteration of phenological timing, distribution patterns and resource availability have the potential to alter trophic level structure as a secondary consequence of the direct effects (Caron & Hutchins, 2013, Peñuelas *et al.*, 2009). As a consequence of climate change, biological changes observed in the phenology of species and populations can act as a feedback mechanism altering community structure and ecosystem stability (Nakazawa & Doi, 2012).

To assess the impact of climate change on the marine ecosystem, long-term ecological studies such as the CPR, and numerous other time series can be used to explore plankton phenology. In the following section, a long-term coastal, Mediterranean time series is introduced as the basis for the work carried out in this thesis to identify phenology in plankton.

1.3 The Long-Term Ecological Research at Station MareChiara (LTER-MC)

The Gulf of Naples (GoN) is a coastal sub-basin of the Tyrrhenian Sea, in the Western Mediterranean. The diverse plankton communities of the GoN sustain a local fishing industry for the densely inhabited city of Naples and the Campania Region; thus, the GoN has an important environmental and economic role within the Mediterranean (Cianelli *et al.*, 2011) Since the establishment of the Stazione Zoologica Anton Dohrn (SZN) in 1872, the marine flora and fauna of the GoN have been sampled and analyzed. The earliest records of marine pelagic copepods in the GoN date back to the studies of Giesbrecht (Giesbrecht W., 1893), and Lo Bianco (Lo Bianco, 1903). In addition to plankton studies, SZN is the location of Europe's oldest aquarium, which was established in 1874 and still maintains both live and preserved specimen collections of native species from the GoN (http://www.szn.it).

The GONEP (Gulf Of Naples Ecological Program), initiated in 1975, represented the first comprehensive investigation towards an understanding of the hydrography and biology of the GoN (Carrada *et al.*, 1981, Carrada *et al.*, 1980, Scotto di Carlo & Ianora, 1983). Additional campaigns in later years surveyed the Gulf in the frame of various national programs (CASMEZ, ALICI, RED-TIDE). A regular time-series of plankton investigation was established in January 1984 at a fixed site, Station MareChiara (stn MC), in the inner GoN. The aim of this ongoing time-series is to characterize the structure and function of the planktonic system and their modes of temporal variability. In 2006, a peer review assessment of the time series established stn MC as a Long Term Ecological Research station (LTER-MC) within the framework of the European and International LTER network.

Since the establishment of a fixed station and monitoring program, plankton research at the Stazione Zoologica has included the study of temporal dynamics of plankton and environmental parameters, Scotto di Carlo et al., 1985; Ribera d'Alcalà et al.,

2004) and seasonal patterns of phytoplankton and zooplankton (Zingone et al. 1990, 1995, 2010; Modigh 2001; Ribera d'Alcalà et al. 2004). Zooplankton community structure and succession have been identified (Mazzocchi & Ribera d'Alcalà, 1995), mesozooplankton life history traits (Ianora, 1998) and population structure and vertical distribution (DiCapua & Mazzocchi, 2004) or seasonal and long-term variability (Mazzocchi *et al.*, 2007) of copepod species. More recently, phytoplankton studies have been carried out at the molecular level to identify specific species (McDonald *et al.*, 2007) and toxic morphotypes (Cerino *et al.*, 2005).

The plankton data at LTER-MC have been recently analyzed in comparative time series studies to characterize seasonal and long term patterns of chlorophyll *a* (Cloern & Jassby, 2010, Zingone *et al.*, 2003), and zooplankton (Berline *et al.*, 2012). Plankton data from LTER-MC have been submitted to the SCOR working groups WG137 (Patterns of Phytoplankton Dynamics in Coastal Ecosystems) and WG125 (Global Comparisons of Zooplankton Time Series). Zooplankton data are included, together with 37 other global zooplankton time series, in the annual report published by the ICES Working Group on Zooplankton Ecology (O'Brien et al. 2010). Station MC is one of six Mediterranean sites established, independently, as plankton observatories that maintain phytoplankton and zooplankton time series. These include the times series in the Balearic Sea (since 1994), in the Bay of Villefranche (Ligurian Sea, since 1966), in the Gulf of Trieste (Northern Adriatic Sea, since 1970), at stn Stončica in the central Adriatic Sea (since 1959), and in the Saroňikos Gulf (Aegean Sea, since 1987).

1.3.1 Study area characterization

The Gulf of Naples (GoN) spans from 40°32'N to 40°50'N and from 13°28'E to 14°52'E, and extends over 870 km^2 with an average depth of 170 m. The LTER-MC station (stn MC) is located in the inner part of the GoN, at 40°48.5'N, 14°15'E on the 80 m isobath at a distance of two nautical miles from the city of Naples (Figure 1). The GoN is influenced by local topography and water exchange between its inner area and the open Tyrrhenian Sea (Carrada et al., 1981). As a result, weather and circulation patterns are established by both local land and basin scale features (Cianelli *et al.*, 2011). These hydrographic features of the GoN are important factors in driving the pelagic system at stn MC.

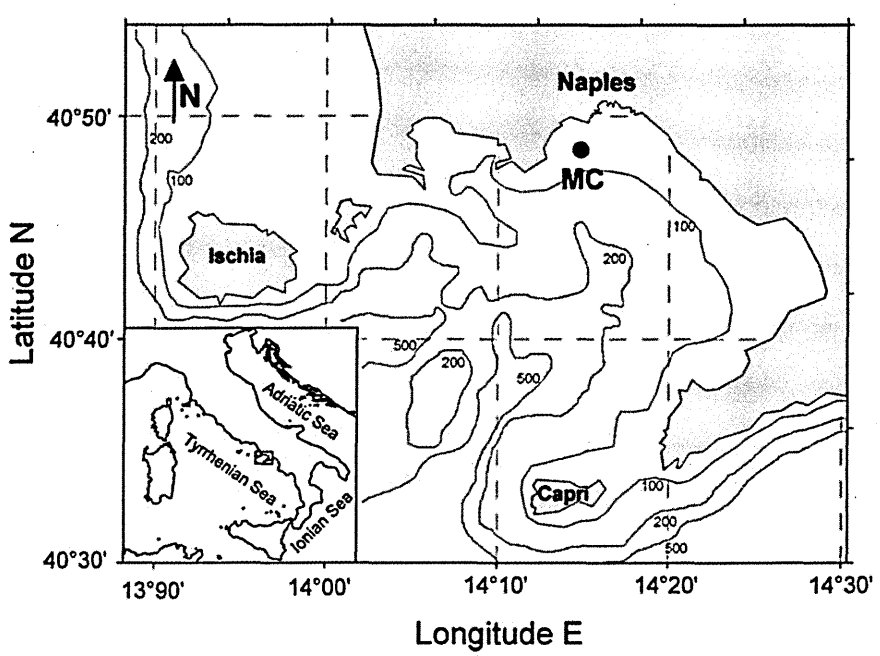

Figure 1.1 Location of the Gulf of Naples Long Term Ecological Research station MareChiara (LTER-MC) indicated by filled black circle. Contour lines indicate isobath depths respectively.

The surrounding topography of Naples is defined by the volcano Mount Vesuvius to the east with an elevation of 1,281m and smaller features such as the hills of Posillipo, Vomero, Camaldoli, and Capodimonte. The Gulf is bordered by the Islands of Ischia and Procida to the north-west, the Sorrento Peninsula and the Island of Capri to the south. Coastal GoN waters receive surface runoff from the heavily urbanized area of the Campania Region coastline and drainage from the nearby Volturno and Sarno rivers, which transport dissolved nutrients, sediment and lower salinity surface waters after periodic precipitation events (Cianelli *et al.*, 2011). The GoN is defined by the surrounding physical and human landscape. This combination of natural and anthropogenic features drives the variability at short and long time scales in the pelagic ecosystem.

Episodic wind and precipitation events combined with local meteorology, such as cloud cover and light penetration, are important processes that drive short term water column variability and the plankton community in the GoN. The GoN has low tidal amplitude and increased surface wave height only as a result of wind driven events (Grieco *et al.*, 2005). The general circulation patterns have been attributed to seasonal wind regime patterns (Cianelli *et al.*, 2011). During winter, wind driven mixing and runoff during periods of winter precipitation contribute to substantial nutrient concentrations and lower salinity. In spring, fresher, nutrient rich surface waters contribute to phytoplankton production, which is sustained and restricted to the stratified upper portion of the water column (Carrada *et al.*, 1980). In summer, more coastal surface waters occupy the GoN and are characterized by higher salinity, stratification and a shallow mixed layer (Carrada et. al, 1980; Hopkins, et al., 1994).

1.3.2. Methods of abiotic and biotic sampling at LTER-MC

Sampling at stn MC began in January 1984, with an interruption from August 1991 to February 1995. Sampling frequency was biweekly until 1991 and has been weekly since 1995. Standard water column parameters, (temperature, salinity, pressure, fluorescence) are recorded from vertical CTD hydrocast profiles. Vertical measurements were made using 5-1L Niskin bottles with reversing thermometers in the years from 1984-1995 and intermittently in the period 1995-2000. From 1995 to present, profiles were collected using a CTD equipped with a vertically mounted SBE911 fluorometer and water samples are collected from twelve 10 L Niskin bottles mounted on a Rosette sampler. The following depths were sampled: 0.5, 2, 5, 10, 20, 30, 40, 50, 60, and 70 m for determination of dissolved oxygen and nutrients.

Dissolved nutrients (NO_2, NO_3, NH_4, PO_4, SiO_4) were measured using a TECHNICON II nutrient autoanalyzer according to the methods of (Hansen & Grasshoff, 1983). Mixed layer depth (MLD) was calculated from discrete density profiles and expressed as the depth at which a change in density ($\Delta\ \sigma\theta$) with respect to the density value at 10 m depth is higher than a threshold of 0.03 kg m^{-3} using the method of (de Boyer Montégut *et al.*, 2004) (Zingone et al. 2010b; V. Saggiomo, personal communication). Samples for determination of chlorophyll *a* (chl *a*) as a proxy of phytoplankton biomass were taken at selected depths (0, 2, 5, 10, 20, 40, and 60 m). From 1984-1991, chl *a* was determined using spectrophotometric methods (Strickland & Parsons, 1972), and from 1995 onwards using a Shimadzu RF-5301PC spectrofluorometer and standard methods (Holm-Hansen *et al.*, 1965). Samples were taken at selected depths for HPLC pigment analysis of phytoplankton composition starting in 1988-2000 and from 2003 until present. Phytopigment analysis is performed using the CHEMTEX software and methods described in (Casotti & Ribera d'Alcalà, 2000). Picoplankton abundance (picoeukaryotes, cyanobacteria and heterotrophic bacteria) has been estimated since 2007. Duplicate 1ml

sample aliquots are collected from each depth are preserved with 0.5 % gluteraldehyde in 1.5 ml cryovials using methods described in (Casotti & Ribera d'Alcalà, 2000). Samples are incubated at ambient temperature onboard and then plunged into liquid nitrogen for temporary storage. Populations are quantified using a Becton Dickinson FACSCalibur flow cytometer.

1.3.2.a Phytoplankton

Phytoplankton samples are collected to determine abundance and taxonomic composition from the 0.5 m surface Niskin bottle and immediately fixed with pH neutral (borate buffered) formaldehyde to a final concentration of 0.8-1.6 % as described in (Ribera d'Alcalà *et al.*, 2004, Zingone *et al.*, 2010a). A live sample is collected from a horizontal surface 20 μm net tow for visual inspection of larger and rarer species. Cell counts are performed using an inverted microscope after sedimentation of variable volumes of seawater (1-100 ml), depending on cell concentration (Utermöhl, 1958), on two transects representing ca. 1/30 of the whole bottom area of the sedimentation chamber at 400X magnification. For selected species, the identification has been checked with an electron microscope. Species level detection of cells smaller than 2 μm is not possible with standard microscopy; however the abundance of autotrophic eukaryotes within this size range is assessed using flow cytometry. Carbon content is determined based on linear size measurements taken over one year of sampling and then, occasionally, on selected samples for species that were rare or variable in size to establish cell biovolume. In the early portion of the time series, carbon content was calculated using the formula introduced by (Strathmann, 1967). At present, carbon content is calculated from a mean cell biovolume using standard conversion formulas in (Menden-Deuer & Lessard, 2000). Through combined microscopic counts and identification, phytoplankton are quantified and placed into functional groups composed of diatoms, dinoflagellates, coccolithophores and established size class groupings (Zingone & Sarno, 2001).

1.3.2.b Microzooplankton

Microzooplankton samples were collected from the 0.5 m Niskin bottles in 1984 and from September 1996 onwards, with an interruption in the first half of 2000. Although formaline as a preservative causes loss of naked ciliates as compared to Lugol's solution, a neutral buffered solution of borate formaldehyde (1.6 % final concentration) was chosen as a fixative as it permits the distinction of chloroplasts within mixotrophic species. Ciliates from subsamples of variable volume (25-250 ml) were counted over the whole bottom of the sedimentation chamber scanned at 340X magnification with an inverted microscope. For biomass calculations, linear dimensions of the ciliate cells were measured in all samples and biovolumes calculated by reference to simple geometrical shapes. Ciliate carbon content was calculated using the conversion factor of 0.14 pg C μm^{-3} which takes into account cell shrinkage caused by formol fixation (Putt & Stoecker, 1989).

1.3.2.c Mesozooplankton

Mesozooplankton samples are obtained from two vertical tows in the upper 50 m layer with a Nansen net (200 μm mesh, 113 cm mouth diameter). One live sample is used to measure biomass as dry weight (mg m^{-3}), and a second sample is fixed with buffered formaldehyde (2-4 % final concentration) for taxonomic identification and counts. Aliquots ranging from 1/4 to 1/32, according to sample density, were taken by using the Huntsman beaker subsampling technique (Van Guelpen *et al.*, 1982) in the early portion of the time series and, in recent years, by using a large-bore graduated pipette (Mazzocchi *et al.*, 2011). In these aliquots, all zooplankton are identified and counted, while the rest of the sample is checked for the presence of rare species. Adult copepods are separated by gender for most species; males are identified to genus level for *Clausocalanus*, *Calocalanus* and *Oithona*, and at family level for Oncaeidae and Corycaeidae. Juveniles (from CII or CIII according to species are efficiently retained by 200 μm mesh) are identified at the species level in most cases, or grouped at genus or family level (e.g.

Calocalanus, *Oithona*, Oncaeidae, Corycaeidae). Taxonomic abundance counts of *Clausocalanus* spp. juveniles, and *Paracalanus parvus* were pooled together during the first years of this series due to taxonomic uncertainty at species level. Among other zooplankters, some groups were identified at the species level during most years of the series (e.g. cladocerans, chaetognaths, siphonophores), while others were always identified at a higher taxonomic level. At present, zooplanktonic organisms are not routinely identified by size; however a size structured analysis of zooplankton was the study of a PhD thesis using measurements made using the ZooScan system (Garcia-Comas Rubio, 2009).

1.3.3. The pelagic ecosystem at LTER-MC

1.3.3.a The abiotic environment

The environment at station MareChiara has a strong seasonal cycle and a relatively wide inter-annual variability. The seasonal cycle divided by calendar months as winter (JFM), spring (AMJ), summer (JAS), and autumn (OND) varies between periods of instability (when locally strong wind or heavy precipitation events introduce runoff and mixing), to calm periods when sunlight favors slight warming and stabilization of surface waters. During spring, water column conditions transition from a well mixed to a gradually stratified, stable water column. Summer (July-September) conditions represent a shift from a humid spring to hot Mediterranean weather through late August (Lionello, 2006). The late autumn phenomenon known as Indian summer or locally as "St. Martin's Summer", occurs when mild, dry high pressure, and stable light and calm water column conditions persist for several days (Zingone *et al.*, 1995). This environmental feature is characteristic of Northern Hemisphere regions south of 50°, and results in clear, calm conditions with higher light which supports favorable growth periods for phytoplankton

Abiotic parameters at stn MC (temperature, mixed layer depth -MLD, salinity, oxygen, and nutrients) have clear seasonal patterns and high inter-annual variability

primarily driven by local meteorological events (Ribera d'Alcalà et al. 2004). The lowest annual temperatures at stn MC are recorded in March and the highest in September and October. Depth integrated temperature (between 0-50 m) in the years 1984-2006 ranged from 13.68 to 15.50 °C in winter (Jan.-March), 14.75-17.53 °C in spring (April – June), 18.26-21 °C in summer (July-Aug.), and 18.20-20.24 °C in Autumn (Sep.-Dec.) (Mazzocchi *et al.*, 2011). In years 1996 and 1998, the average depth integrated temperature in autumn was lower (16.35°C), whereas in 1987 lower than average spring (14.75°C) and warmer than average autumn temperature (20.24°C) were recorded (Mazzocchi *et al.*, 2011).

Seasonal stratification of the water column begins in late March and lasts through October. On average, the inter-annual MLD is above 30 m however it varies seasonally. The spring increase in temperature creates a shallower MLD above 10 m that is stable between May and August (Mazzocchi *et al.*, 2011). During the central portion of the time series spanning 1995 to 1999, the MLD had similar annual mean values, whereas the MLD was deeper and more variable in later years (2000-2006). In 2002, the average MLD was shallower (10m) than in other years (Mazzocchi *et al.*, 2011).

Depth integrated salinity values between 0 and 70 m from 1984-2006 were highest in November-December (37.98 psu) and lowest in May (37.84 psu) (Mazzocchi *et al.*, 2012). Annual surface salinity values (1984-2006), ranged from 37.28 to 38.29 psu with an average range of 37.87 $\pm$0.21 in winter and 37.68$\pm$0.35 psu in non-winter periods (Zingone *et al.*, 2010b). During the first three years (1984-'86), the lowest salinity values due tocoastal runoff were recorded. In 1986 and 1997, surface temperature and salinity values corresponded with lateral advection from runoff and precipitation events. The salinity increased during the following four years, and then it slightly decreased again in 1996-1998 (Ribera d'Alcalà *et al.*, 2004).

Discrete and depth integrated oxygen is nearly always completely saturated throughout the water column due to the combined effect of shallow depth, and mixing. Nutrients are never completely depleted within the water column (Ribera d'Alcalà et al. 2004). Peak nutrient concentrations occur within the first few months (Jan-Mar) and decline between Apr-Aug. when they are consumed by phytoplankton. Total dissolved inorganic nitrogen (TIN) values remain constant at an average of 2 μmol dm^{-3}. During summer, TIN concentration is lower in the upper layer (0-10 m). On average, nitrates and silicate concentrations are higher during the winter (Zingone *et al.*, 2010b). Surface TDN (data from 1984-2006) was 3.44+ 2.69 μmol L^{-1} whereas depth integrated values were lower at 1.84 + 0.98 μmol L^{-1} (D' Alelio *et al.*, 2010). Over the long term, nutrients did not exhibit a clear inter-annual trend. Nutrients were highly variable from year to year in both the 0-10 m and 10-70 m layers. Lower than average values in some years of the second part of the series (years 1995, 1996 and 1999) were detected for TIN, SiO_4 and PO_4 (Ribera d'Alcalà *et al.*, 2004).

1.3.3.b Phytoplankton

The phytoplankton community at stn MC is highly diverse and more than 600 species have been taxonomically identified as a result of field and laboratory based studies at SZN. The LTER-MC time series data set includes only 337 total species for which abundance estimate exist and includes 124 diatom species (centric and pennate), 117 species of dinoflagellates (thecate and athecate), 47 species of coccolithophores, and 56 phytoflagellate species. Taxonomic identification to genus level and species (when possible) is carried out with the exception of small flagellates below 2μm in size. The most abundant phytoplankton classes at stn MC are diatoms and small flagellates (average size 3.6μm).

Diatoms are dominated by several key species; *Chaetoceros tenuissimus*, *Skeletonema pseudocostatum*, *Leptocylindrus danicus*, *Chaetoceros socialis*, and

Minidiscus comicus which comprise 39.5+5.8% of the annual biomass (Ribera d'Alcalà et al. 2004). The Pseudo-nitzschia genus is composed of 25 different pennate, colonial marine diatoms some of which have more recently been found to contain the neurotoxin domoic acid (Cerino, 2006, fl-d107). In early studies of the phytoplankton community in the Gulf of Naples, this species was referred to as 'colonial Nitzschia' but with the identification of 7 morphologically similar species in 1996, the identification was changed to *Pseudo-nitzschia.* Within the *Pseudo-nitzschia* group several species are identified including *Pseudo-nitzschia galaxiae, delicatissima, fraudulenta/subfraudulenta, multistriata, pseudodelicatissima, subpacifica,* and *Pseudo-nitzschia* spp. In 2 species, *P. galaxiae* and *P. multistriata* were found to contain low concentrations of the toxin; domoic acid, however toxicity was not analyzed until 2001 and more recently, molecular analysis has been used to discriminate genetically different strains (Tesson, PhD thesis, 2010). In addition to this species, the highly abundant diatom *Skeletonema* is composed of 5 dominant species including *Skeletonema dohrnii, menzelii , pseudocostatum, tropicum ,* and spp. of which *Skeletonema pseudocostatum* is the most abundant (Kooistra *et al.,* 2008). Using light and electron microscopy, sequence analysis of the hyper-variable region of nuclear LSU rDNA in *Skeletonema* species revealed that all recently described species were genetically distinct and only 2 were morophologically distinct (Kooistra *et al.*, 2008).

Dinoflagellates at stn MC are identified to species based on armored or unarmored morphologies. Several species are important at stn MC. *Calciodinelloideae* n.d., *Protoperidinium* spp., and *Heterocapsa niei,* and *Prorocentrum triestinum* contribute to blooms (Ribera d'Alcalà et al. 2004). The resting stage cysts of some dinoflagellates have been attributed to period toxic blooms in many coastal environments. A systematic 2 year study to identify and quantify dinoflagellate cyst production at stn MC identified 59 morphotypes using benthic sediment corer and bottom grab sampling (Montresor *et al.,*

1998). The sediment composition at stn MC and other surrounding sampling stations within the 100 m isobaths were primarily sand-silt, however at depths outside the shelf, the sediment composition was finer and predominantly silt or silt-clay. Station MC is characterized by calcareous Peridiniales cysts. In particular the species *Scrippsiella trochoidea* dominates the dinoflagellate assemblages in the sediments, contrasting with deeper stations that are dominated by organic non-calcareous Peridiniales of the genus *Protoperidinium* spp.. Additionally, resting cysts of potentially toxic dinoflagellate species *Alexandrium andersoni* and *Gymnodinium catenatum*-like species are found, although their mobile stages have never been recorded in the GoN plankton. In addition, *Alexandrium minutum*, *Akashiwo sanguinea* and *Prorocentrum minimum* are at times recorded, but no harmful blooms have occurred in the GoN.

A diverse number of coccolithophores are present, however only a few contribute to the total abundance. *Emiliania huxleyi* and *Calciopappus caudatus* are two dominant species found throughout the year (Ribera d'Alcalà et al. 2004). Phytoflagellates are an important phytoplankton group; however no key species have been attributed to blooms or features at stn MC. In more recent years of the time series, phytoflagellate abundance has increased while the overall size spectrum of the phytoplankton community has decreased (SCORwg137, 2011 unpublished and personal communication with D. Sarno and A. Zingone).

The picoplankton fraction of the phytoplankton community has been identified using flow cytometry since 2007. The dominant picocyanobacterial populations *Prochlorococcus* and *Synechococcus* as well as picoeukaryotes are enumerated. The presence of different picoplankton populations in the GoN were attributed to light adaptation strategies and mesoscale water mass features by using nutrient data coupled with HPLC and picoplankton analysis to identify offshore deeper Tyrrhenian Sea water (Casotti & Ribera d'Alcalà, 2000).

1.3.3.c Zooplankton

The zooplankton community at stn MC includes microzooplankton and mesozooplankton. Microzooplankton are a critical link in the trophic food web as a food source for mesozooplankton and as grazers of small phytoplankton and bacteria. This group includes autotrophic and mixotrophic ciliated protozoa, tintinnids, heterotrophic dinoflagellates as well as copepod nauplii (Modigh & Franzè, 2009). The microzooplankton at stn MC is dominated primarily by small (<30 μm), naked choreotrich ciliates. Standing stock analysis of ciliated protozoan samples revealed that photosynthetic ciliates comprised 49% of the total ciliate biomass; 25% was due to heterotrophic naked choreotrichs and 16% was due to tintinnids (Modigh, 2001). Tintinnids contribute on average 10% to annual ciliate abundance (Ribera d'Alcalà et al. 2004). A small portion is completely autotrophic and includes the species *Mesodinium rubrum*, while the remaining assemblage is composed of a diverse assemblage of mixotrophic species such as *Strombidium* spp. (Modigh, 2001). Tintinnids are composed of 57 species with more recent estimates at more than 80 (Ribera d'Alcalà et al. 2004), however only 7 dominant species account for 81% of the total abundance including; *Tintinnopsis minuta*, *T. beroidea*, *Metacylis annulifera*, *Eutintinnus tubulosus*, *Helicostomella subulata*, *Salpingella curta* and *S. decurtata* (in rank order) (Modigh, 2001, Modigh & Castaldo, 2002). Overall diversity of tintinnid populations ranged between 0 and 3.2 with a mean of 1.5 + 0.7 stdev using the Shannon Diversity index.

The mesozooplankton community is dominated by copepods (including calanoid, cyclopoid and harpactacoid orders) which comprise 61-78% of the total mesozooplankton annual abundance; followed in rank order of abundance by cladocerans, tunicates, meroplankton, cnidarians, chaetognaths and ostracods. Tunicates and meroplankton are primarily represented by appendicularians and decopod larvae, respectively (Mazzocchi *et al.*, 2011). Copepods are dominated by most small (<1 mm) sized species that include

juveniles of *Clausocalanus* spp./*Paracalanus parvus*, followed by other taxa in rank order of importance: *Acartia clausi*, *Oithona* spp. (nine species), *P. parvus* adults, *Clausocalanus* spp. adults (eight species), *Centropages typicus*, *Temora stylifera*, oncaeids, *Calocalanus* spp. (nine species), and *Ctenocalanus vanus* (Mazzocchi *et al.*, 2011). Despite a wide taxonomic diversity of zooplankton, several copepod species including *Acartia clausi, Centropages typicus*, *Temora stylifera*, and *Paracalanus parvus* maintain stable long term cycles of abundance at stn MC (Mazzocchi et al., 2011b).

1.3.4. Seasonal patterns of plankton assembly

The annual cycles of plankton biomass and composition at stn MC reflect the influence of coastal GoN waters during late spring and summer and off-shore Tyrrhenian Sea water during winter. Spring phytoplankton biomass measured as bulk depth integrated (0-70m) chlorophyll reaches 0.97 $\mu g\ L^{-1}$ in March, followed by two successive peaks in May and October where values are 0.77 $\mu g\ L^{-1}$ and 0.67 $\mu g\ L^{-1}$ respectively (Mazzocchi *et al.*, 2011). Phytoplankton abundance reaches peak values of $20x10^6$ cells L^{-1} in the integrated 0-60 m layer and $10x10^6$ cells L^{-1} at the surface (Ribera d'Alcalà et al. 2004). Accumulated winter biomass results in a late winter average chlorophyll a peak of 0.97µg chl *a* L^{-1} and average phytoplankton concentrations of $1.2x10^6$ cells L^{-1} (Zingone *et al.*, 2010a). Average annual abundance is below $5x10^6$ cells L^{-1} between November through March. Because phytoplankton are quantified from a surface depth sample, depth integrated values over the year reflect a bimodal peak rather than a single peak in spring.

The phytoplankton community at stn MC has regular annual patterns of succession from a phytoflagellate dominated community in winter to bloom forming colonial diatoms in early spring (and at times also in winter) to non-colonial small chained or single celled diatoms in summer, when dinoflagellate populations also increase. The highly variable environment at stn MC does not allow the phytoplankton community to develop a typical

early to late pioneer stage succession; completely dominated by dinoflagellates, rather it is characterized by a state of continual renewal and re-establishment of species assemblages whereby diatom populations play a major role in all seasons (Zingone et al. 1995; Ribera d'Alcalà et al. 2004). Generally, the phytoplankton community in May is dominated by diatoms with a large contribution due to dinoflagellates in May and June. During the year, phytoflagellates are also numerous in May. In spring, warming surface waters and lower salinity waters contribute to a shallowing of the mixed layer depth (<10m) with a shift of the dominant phytoplankton community from colonial forming to single celled diatoms comprised of *Chaetoceros tenuissimus*, and *Skeletonema pseudocostatusm* and dinoflagellates (*Prorocentrum triestinum*, and *Heterocapsa nieii*). The transition from summer to autumn marks an increase in coccolithophorids and a second bloom during this period. The winter phytoplankton community is dominated by small flagellates most of the time, but blooms of larger colonial diatoms such as *Chaetoceros socialis*, *Thalassiosira* spp., and *Leptocylindrus danicus* also occur, especially in the first part of the time series (Zingone *et al.*, 2010b). Other species such as the abundant diatom, *Skeletonema dohrnii* occur only during winter and spring in the GoN (Kooistra *et al.*, 2008).

The presence of high viral infection in bloom forming species has been attributed to viral-mediated mortality and eventual bloom collapse in some cases (Suttle, 2007).

The seasonal abundance of an autumn to late spring picoeukaryotic bloom forming prasinophyte *Micromonas pusilla* at stn MC was investigated over one annual cycle and over the spring cycle for three consecutive years. Viruses were present throughout the year at concentrations of 0.02-1.9x10^3 viruses mL^{-1}, but ever exceeded abundance of the *M. pusilla* population (Zingone *et al.*, 1999a). This study of *Micromonas* pusilla was one of the first to investigate host-viral dynamics of a picoeukaryotic species at stn MC. Results concluded that although viral populations were high and at times ranged from between 2.3

to 13% of the *Micromonas* population, it did not result in the decline or collapse of the seasonal bloom (Zingone *et al.*, 1999b).

The seasonal cycle of mesozooplankton abundance and biomass reaches maximum values in spring (April-May) and summer (July-September) with maximum abundances recorded at 11,148 ind. m^{-3} in 1990 and minimum values in winter (December and January) recorded at a minimum of 2625 ind. m^{-3} (Mazzocchi and Ribera, 1995; Ribera d'Alcalà et al. 2004). In winter, mesozooplankton is dominated by small-sized copepod genera consisting of *Clausocalanus*, *Calocalanus*, *Oithona* and *Oncaea*. In winter-early spring large off-shore calanoids appear in coastal waters (Mazzocchi et al., 2011b). The summer zooplankton community is marked by the sudden appearance and dominance of cladocerans (Mazzocchi & Ribera d'Alcalà, 1995). The species *Penilia avirostris* has a peak in July-August and co-occurs with the small copepod *Paracalanus parvus* which is seasonally restricted to summer.

During autumn (Oct-Nov), an enhanced period of phytoplankton biomass and second bloom period favors an increase in copepod abundance. Four abundant copepod species peak in regular seasonal succession starting with: *Acartia clausi* and *Centropages typicus* in spring-early summer, *Paracalanus parvus* during summer-early autumn and *Temora stylifera* in autumn (Mazzocchi et al., 2011b). A model simulation was used to assess the impact of diet on the copepod *Temora stylifera* (Mazzocchi *et al.*, 2006) and found that a mixed diet consisting of the diatom (*Thalassiosira rotula*), and dinoflagellate (*Prorocentrium minimum*) resulted in high population increases. Current doctoral research carried out by Mahadik, unp. May reveal more insight into species-specific grazing dynamics. In addition to copepods, appendicularians have the highest relative abundance and meroplanktonic larvae peak in abundance during winter (Ribera d'Alcalà et al. 2004).

Microzooplankton ciliate populations are dominated by mixotrophic choreotrichs during spring and summer, and autotrophic ciliate populations in winter. The highest

ciliate abundance and biomass was measured during stratified water column conditions (Modigh, 2001). The average annual biomass of ciliates over a 3 year period (1997-1999) ranged from 10.5 μg C l^{-1} in 1999 to11.8 μg C l^{-1}. Mixotrophic choreotrichs were present during stratified conditions and contributed up to 89% of the total ciliate biomass. Photosynthetic ciliates had seasonally varied contributions to total ciliate biomass. The autotrophic ciliate *Mesodinium rubrum* contributed on average 22% to 48% of the total ciliate biomass. This dominant species had small (28 μm) and large sized (45 μm cell length) forms which both showed high abundance in spring and autumn. The seasonal cycle of ciliates was characterized by the succession of three dominant mixotrophic genera. The mixotrophic species *Laboea strobila* and *Cyrtostrombidium* sp. were present in early spring (March-April) followed by *Tontonia* spp. which reached maximum abundance during late summer and autumn when *Laboea* was not present. A diversified assemblage composed of mixotrophic *Strombidium* was present until late autumn.

The plankton communities at stn MC exhibit a strong seasonal cycle and high inter-annual variability. The long term signature of plankton assemblages has been considered a stable feature with recurrent seasonal patterns in both phyto and zooplankton (Ribera d'Alcalà et al. 2004; Mazzocchi et al., 2011b). Some years within the time series show variability within the plankton system. The years 2004 and 2006 were particularly peak years of zooplankton species abundance (Mazzocchi *et al.*, 2011). In 2004, the highest annual average temperature (surface) and lowest corresponding salinity values were recorded. Higher chl *a* concentrations were measured in the early portion of the time series between 1989 and 1990 and showed a decreasing trend starting in 1995 (Ribera d'Alcalà et al. 2004). Along with the noticeable decline in chl *a*, higher abundance of cells attributed to small-sized phytoplankton cells has been measured over the long term. (Ribera d'Alcalà et al. 2004). This feature suggests that a potentially larger trend can be observed in this time series. In the early portion of the time series between 1984 and 1988, productivity

and high autotrophic biomass was attributed to coastal nutrient loading in 1985 (Zingone *et al.*, 1995). After the year 2000, dinoflagellates have shown a greater role in the winter phytoplankton community (Zingone *et al.*, 2010b). In recent years, the diatom *Pseudo-nitzschia galaxiae* has been found in high abundance in February–March (up to 7.3×105 cells l−1 in March 1996). Annual peak concentrations generally occurred in May and August (up to 9.4×106 cells l−1 May 1985) generally occurred in May and August (Cerino *et al.*, 2005).

Mean annual zooplankton abundance at stn MC had higher inter-annual variability during the first portion of the time series between 1984 and 1990 in contrast to later years between 1995 and 2006 In the long term (1984-2006), some abundant copepod species maintained stable, regular inter-annual patterns of succession, though with some variability in their patterns of abundance (Mazzocchi *et al.*, 2011). *Paracalanus parvus* had a peak abundance in 1984, lower abundance in subsequent years and a gradual recovery in more recent years. The copepod *Temora stylifera* had the lowest inter-annual variability over the long term, however from 1988 until 2001, a steady decrease in abundance was observed. *T. stylifera* had a minimum abundance in 2001 and recovered to reach maximum abundance in 2004. A significant decrease in summer abundance was recorded for *A. clausi* and *C. typicus* (Mazzocchi et al., 2011b). The copepod *Centropages typicus* showed remarkable inter-annual variability in its seasonal peaks. Greater abundance of *C. typicus* was recorded in the early portion of the time series whereas after 1995, the population only reached similar high abundance in years 1999, 2000 and 2004. In previous years evaluated 1995-1999, the peaks were on average in July and in 1999, in August, whereas in 2000, the peak was recorded much earlier in April with a second peak in mid June a slightly earlier decline in September (Mazzocchi *et al.*, 2007).

Some notable inter-annual changes have been observed in the occurrence of a few rare copepod species (Mazzocchi *et al.*, 2011). The late winter-early spring *Acartia*

margalefi has disappeared and abundance of the summer *Paracartia latisetosa* has declined. The spring *Diaixis pygmaea* shows a decline in abundance from the year 2000-2006. In contrast, the late summer-early autumn species *Paracartia grani* has appeared only after 1995 and is now regularly present at stn MC. Additionally, the autumn-winter species *Acartia negligens* was first recorded starting in 1998 (Mazzocchi *et al.*, 2011). The persistence of key species and in some cases, the unique disappearance of others (eg. *Acartia margalefi*) within the dominant functional groups such as copepods is a feature particular to the zooplankton community at station MC. Despite a positive trend in increasing summer temperature, and decreasing chl *a,* copepod assemblages have remained stable over the long term (Mazzocchi, 2012, 2011b). This feature of the zooplankton communities has been attributed to biological plasticity and specifically, functional diversity within copepod assemblages.

Mesozooplankton community structure in the period 1984-2006 was analyzed to identify species associations and homogenous periods that were characterized by specific assemblages (Mazzocchi et al., 2011a). Five taxonomic associations (A1-A5) and five seasonal time periods (P1-P5) were identified throughout the time series. The species association A1 was characterized by the abundant copepods *Acartia clausi* and *Centropages typicus* that contributed to peak abundance from April to July. A2 had a narrow seasonal cycle from June to October with peak abundance in August due to cladocerans. The A3 association had the lowest annual abundance and was almost restricted to the winter months. A4 abundance increased slowly from May to July, and reached a peak in October, then almost disappeared in December. A5 was the most abundant association and had an extended seasonal cycle. This group was comprised primarily of winter zooplankton and had two distinct peak periods, in March – April and in August. A5 also included meroplankton (such as echinoderm and Cirripede larvae) that peak in winter, and decapod larvae that peak in spring as well as copepod species found in

off-shore waters (Mazzocchi et al., 2011a). At an annual scale, the summer (A2) and autumn (A4) species association had a clear seasonal cycle. The identified homogeneous periods were a winter-early spring period (P1), a mid-spring to early summer period (P2), a mid-summer (P3), late summer (P4), and autumn period (A5). In general, zooplankton assemblages were composed of species that were strongly associated in different seasons. The strongest associations that occurred between summer and autumn assemblages were shaped by seasonal forcing. The biological and physical mechanisms that may drive these associations include the position of stn MC within a larger climate regime as well as the temporal variability of environmental conditions.

In review, previous studies of the Gulf of Naples pelagic ecosystem suggest that stn MC has features of both coastal and deeper off-shore waters. A long-term decline in phytoplankton biomass (measured by chlorophyll) as well as recorded occurrences of offshore mesozooplankton taxa supports the current argument that the GoN waters may not fit the typical coastal definition. Furthermore, despite that nutrient replete conditions, phytoplankton biomass does not reach concentrations typically observed in productive coastal ecosystems or otherwise, what would be expected given the favourable light, nutrient and temperature conditions. Species-level studies and long-term monitoring at stn MC, have elucidated the life history patterns of a few key species, however there are still existing gaps in knowledge.

While the observed variability in plankton populations has typically been attributed to physico-chemical mechanisms, the intrinsic mechanisms responsible for the extremely regular composition of species assemblages are not known. At present, the mechanisms responsible for the seasonal patterns of phytoplankton growth and reproduction at GoN are a focus of species-level modeling efforts, but they lack validation from long-term records of in-situ data. Furthermore, to date, no predictive models based on individual traits (commonly called IBM or Individual Based Models) have developed from the existing

time series data. In addition to these unresolved areas, we lack knowledge about the biological response to predicted changes in regional and global climate regimes. If the observed patterns and trends in plankton and environmental data persist, there is an urgent need to develop an integrated plankton model to assess both species and functional level response to climatic factors.

1.5 Aims of thesis

The occurrence of phenological events can be used to understand the structure of ecological communities and in turn, aid in the prediction of biological response to global patterns of climate change. The overall goal of this work is to identify the most important aspects of phenology in the planktonic system using an expanding set of data analysis and statistical modeling tools to explore the long-term patterns at species and community level.

The major aims of this work are to systematically identify long-term patterns in phenology of phytoplankton and zooplankton populations using a species-specific approach. To build upon the rich taxonomic understanding of plankton in the Gulf of Naples, the phenology of individual taxa will be explored to identify long-trends in phenology. To address the possible mechanisms underlying the observed seasonal patterns and long-term changes (if any), an integrated approach will be used to correlate temporal patterns of phenological events with the most important possible environmental drivers.

In addition to a synthetic view of plankton phenology, this work is aimed at assembling an integrated view of changes in phenology. In addition to these aims, the results of this work present the first comprehensive study of phenology in phytoplankton and zooplankton populations in the Gulf of Naples. With a combined knowledge of species-level phenology and ecosystem function, future work is aimed at an integrated model of plankton phenology.

Chapter 2

MATERIALS AND METHODS

2.1 Description of datasets

To analyze the patterns of plankton phenology and their long-term changes at stn. LTER-MC over a 23 year period between 1984 and 2010, two distinct abundance datasets were considered, which initially included 344 phytoplankton and 111 zooplankton categories, respectively. These two datasets corresponded to the extension from 2007 to 2010 of those previously analyzed for investigating other aspects of phytoplankton (Zingone, 2010, 2010) and zooplankton communities (Mackas *et al.*, 2012, Mazzocchi *et al.*, 2011) at stn. LTER-MC.

In particular, data of phytoplankton concentration (cells ml^{-1}) at the surface (0 m) were organized in a matrix of 344 categories x 915 raw observations, and subsequent matrices of abundance x monthly and yearly scales. Phytoplankton categories were organized into four levels; diatoms, dinoflagellates, coccolithophores and other flagellates to maintain the original taxonomic categories for each respective species or group. Individual species within each respective trophic level were then selected for different parts of the analysis. Within each level, taxa that were not identified to species level were placed within a *Genera* level "spp" category. This clarification differs from zooplankton because it is not a sum of different species belonging within genera, but rather signifies an unidentified (at species level) organism within a genus. Additionally, undetermined size classes were preserved from the original taxonomic list to address relevant ecological questions at community-scale. These size categories included undetermined phytoflagellates, cryptophytes, centric and pennate diatoms, naked and thecate dinoflagellates.

Data of zooplankton abundance (ind. m^{-3}) were organized in a matrix of 111 taxonomic categories x 806 raw observations over the whole period 1984-2010. The zooplankton categories were heterogeneous in terms of taxonomic composition, from species to families for the most abundant groups (e.g., copepods, cladocerans), from genus to class level for the less abundant or rare groups. In some cases (e.g., appendicularians,

salps, chaetognaths, siphonophores, meroplanktonic larvae, and a few copepod genera), the identification was limited to higher-than-species level during the first years of the time-series and, for the present analyses, this level was considered for maintaining homogeneity throughout the whole period. In other cases, when species belonging to the same genus (e.g., *Candacia*, *Euchaeta*, *Lucicutia*, *Vettoria*, etc.) or the same family (e.g., Eucalanidae for *Eucalanus*, *Subeucalanus*; Pontellidae for *Pontella*, *Anomalocera*, *Labidocera*) were rare and with negligible abundance, they were grouped at the genus or family levels to be maintained in the analysis, with the assumption that they share similar biological and ecological traits.

Due to a lag of four missing years in the time series between 1991 and 1994, three different periods were considered for the present analyses: Complete time series: 1984-2010; Part I: 1984-1990; Part II: 1995-2010. Seasons were considered according to the calendar, i.e., January, February, March for winter; April, May, June for spring; July, August, September for summer; October, November, December for autumn.

2.2 Selection of plankton taxa

The analyses on plankton phenology were conducted on a selected portion of the two datasets presented above, to include the major and representative parts of phyto- and zooplankton communities, excluding the very rare components. Objective criteria were applied, starting from the rank abundance and frequency curves for a preliminary depiction of the general patterns of community abundance and species composition (Fig. 2.1).

For the rank abundance, the untransformed abundance values of each taxonomic category were summed over the complete time series and the sums were ranked from the most abundant category (1) to lowest one (344 for phytoplankton, 111 for zooplankton). Rank abundance distributions were performed using the *rankabundance* function in the package *BioDiversityR* (Kindt & Coe, 2005) in the data analysis software R (R, 2011).

The rank frequency was calculated on the percentage of occurrence of each category on the total number of observations (915 for phytoplankton and 806 for zooplankton).

After visual inspection of the curves to determine sharp breaks in the rank profiles, in each dataset only the categories with a frequency of occurrence > 5% were retained. Below this limit, corresponding to ~ 2 samples per year, the occurrence of the category was considered occasional or too scarce to depict its phenology. Therefore, in the phytoplankton dataset 102 categories were retained and 242 categories occurring in ≤ 45 observations were excluded. From the zooplankton dataset, 87 categories were retained and 24 categories occurring in ≤ 34 observations were removed. These categories represented different taxonomic levels, e.g., species, genus, family, class. Moreover, to expand the analysis to the community level, additional bulk categories were also included, e.g., total phytoplankton, diatoms, dinoflagellates, coccolithophores, and other flagellates (not identified to species level), total zooplankton and total copepods. The final selection of plankton taxa included 104 phytoplankton and 88 zooplankton categories (Table 2.1).

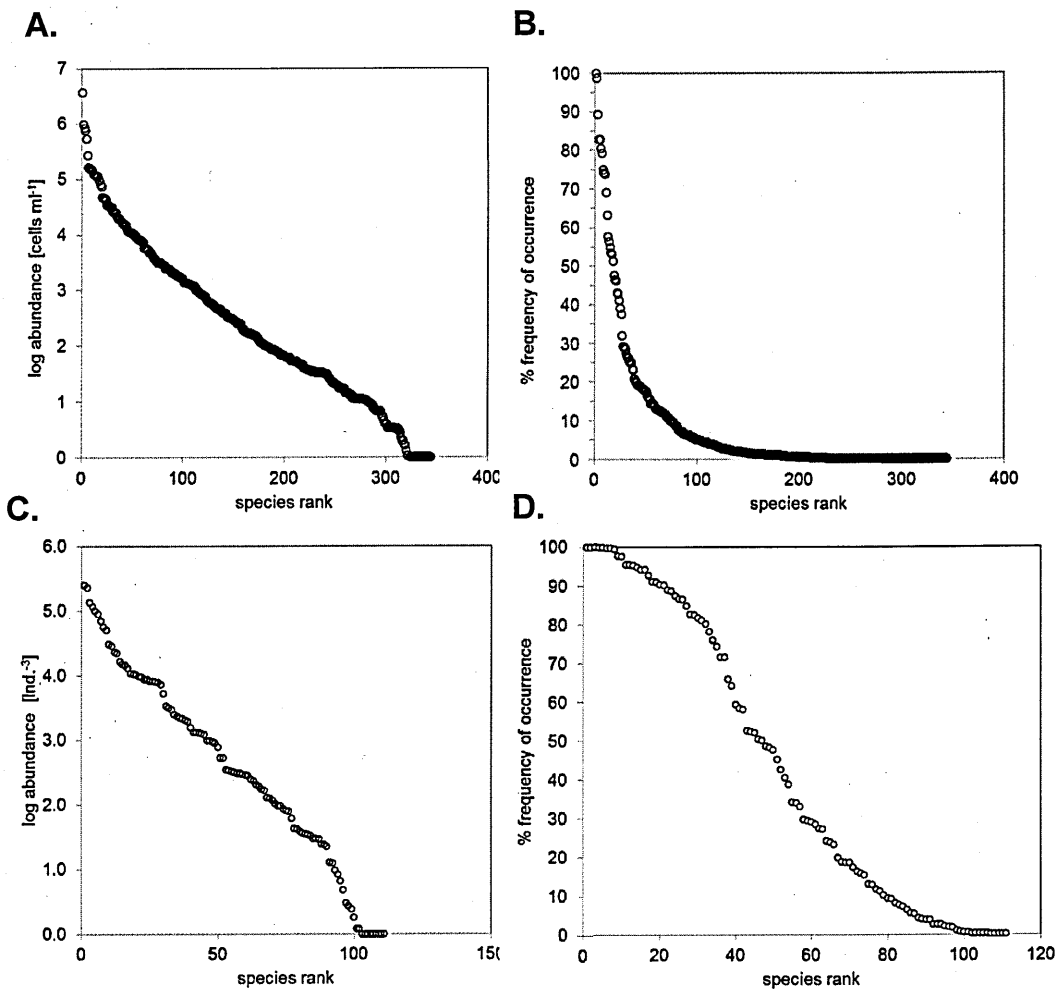

Figure 2.1 Curves of rank abundance (A,C) and rank frequency (B,D) for 344 phytoplankton (a) and 111 zooplankton (b) taxonomic categories at stn. LTER-MC.

Table 2.1 List of 192 selected phytoplankton (n=104) and zooplankton (n=88) taxa used in phenology analysis. Categories are ordered alphabetically within each category.

Phytoplankton n=104	Zooplankton n=88
Bulk categories	**Bulk categories**
Total coccolithophores	Amphipods
Total diatoms	Appendicularians
Total dinoflagellates	Ascidiacean larvae
Total other flagellates	Bivalve larvae
Total phytoplankton	Chaetognaths
	Cirriped larvae
Coccolithophores	Decapod larvae
Acanthoica quattrospina	Doliolids
Algirosphaera robusta	Echinoderm larvae
Calciopappus caudatus	Gastropods (sum of Gastropod larvae + Pteropods)
Calciosolenia brasiliensis	Hydromedusae
Coccolithophores unidentified	Isopod Epicaridean larvae
Coronosphaera mediterranea	Ostracods
Emiliana huxleyi	Pisces larvae+eggs
Ophiaster spp.	Polychaet larvae
Rhabdosphaera clavigera	Salps
Syracosphaera molischii	Siphonophores
Syracosphaera pulchra	Total copepods
	Total zooplankton
Diatoms	Total cladocerans
Asterionellopsis glacialis	
Bacteriastrum furcatum	**Cladocerans**
Bacteriastrum parallelum	*Evadne+Pseudoevadne*
Bacteriastrum spp.	*Penilia avirostris*
Centric diatoms < 5 µm	*Podon+Pleopis*
Centric diatoms > 10 µm	
Cerataulina pelagica	**Copepods**
Chaetoceros affinis	*Acartia clausi*
Chaetoceros anastomosans	*Acartia danae*
Chaetoceros contortus	*Acartia discaudata*
Chaetoceros curvi-curvi	*Acartia margalefi*
Chaetoceros curvisetus	*Acartia negligens*
Chaetoceros diadema	Calanidae juveniles
Chaetoceros lorenzianus	*Calanus helgolandicus*
Chaetoceros minimus	*Calocalanus* spp.
Chaetoceros peruvianus	*Candacia* spp.
Chaetoceros protuberans	*Centropages kroyeri*
Chaetoceros pseudocurvisetus	*Centropages ponticus*
Chaetoceros simplex	*Centropages typicus*
Chaetoceros socialis	*Centropages violaceus*
Chaetoceros spp.	*Clausocalanus arcuicornis* females
Chaetoceros tenuissimus	*Clausocalanus furcatus* females
Chaetoceros throndsenii	*Clausocalanus jobei* females
Cyclotella atomus var. *atomus*	*Clausocalanus lividus* females
Cyclotella atomus var. *gracilis*	*Clausocalanus mastigophorus* females
Cyclotella spp.	*Clausocalanus parapergens* females
Cylindrotheca closterium	*Clausocalanus paululus* females
Dactyliosolen blavyanus	*Clausocalanus pergens* females
Dactyliosolen fragilissimus	*Clausocalanus* spp. + *Paracalanus parvus* juveniles
Dactyliosolen phuketensis	*Clausocalanus* spp. males
Guinardia striata	*Clytemnestra rostrata*
Lauderia annulata	*Copilia* spp.

Leptocylindrus cf. *danicus*
Leptocylindrus mediterraneus
Leptocylindrus minimus
Lithodesmium cf. *variabile*
Minidiscus comicus
Minutocellus polymorphus
Pennate diatoms < 10 µm
Pennate diatoms > 10 µm
Proboscia alata
Pseudo-nitzschia delicatissima complex
Pseudo-nitzschia galaxiae
Pseudo-nitzschia galaxiae "small morphotype"
Pseudo-nitzschia multistriata
Pseudo-nitzschia pseudodelicatissima complex
Pseudo-nitzschia spp.
Rhizosolenia setigera
Skeletonema menzelii
Skeletonema pseudocostatum
Thalassionema bacillare/frauenfeldii
Thalassionema nitzschioides
Thalassiosira cf. *allenii*
Thalassiosira mediterranea
Thalassiosira rotula
Thalassiosira spp.

Dinoflagellates
Calciodinelloideae unidentified
Heterocapsa niei
Lessardia elongata
Naked dinoflagellates < 15 µm
Naked dinoflagellates > 15 µm
Oxytoxum variabile
Prorocentrum triestinum
Protoperidinium bipes
Protoperidinium spp.
Thecate dinoflagellates < 15 µm
Thecate dinoflagellates > 15 µm

Other flagellates
Apedinella spinifera
Chrysochromulina spp.
Dictyocha fibula
Dinobryon coalescens
Dinobryon faculiferum
Diplostauron cf. *elegans*
Eutreptiella spp.
Heterotroph flagellates
Leucocryptos marina
Meringosphaera mediterranea
Ollicola vangoorii
Pachysphaera spp.
Paulinella ovalis
Phaeocystis spp.
Pseudoscourfieldia marina
Pyramimonas spp.
Rhizomonas setigera
Tetraselmis spp.
Undetermined cryptophyceans < 10 µm
Undetermined cryptophyceans > 10 µm
Undetermined phytoflagellates < 10 µm
Undetermined phytoflagellates > 10 µm

Corycaeus spp.
Ctenocalanus vanus
Diaixis spp.
Eucalanidae
Euchaeta spp.
Euterpina acutifrons
Farranula rostrata
Haloptilus spp.
Harpacticoid undetermined
Heterorhabdus papilliger
Isias clavipes
Lucicutia spp.
Macrosetella gracilis
Mecynocera clausi
Mesocalanus tenuicornis
Microsetella spp.
Nannocalanus minor
Neocalanus gracilis
Oithona atlantica females
Oithona decipiens females
Oithona longispina females
Oithona nana females
Oithona plumifera females
Oithona setigera females
Oithona similis females
Oithona spp. males + juveniles
Oithona tenuis females
Oncaeidae
Pachos punctatum
Paracalanus denudatus
Paracalanus nanus
Paracalanus parvus (only adults)
Paracartia grani
Pleuromamma spp.
Pontellidae
Pontellina plumata
Sapphirina spp.
Scaphocalanus spp.
Scolecithricella spp.
Scolecithrix spp.
Temora stylifera

2.3 Analysis of plankton phenology

From the selected 192 taxonomic categories presented in Table 2.1, a matrix of monthly averaged phytoplankton concentrations and zooplankton abundances was built to show the average annual cycles in three different periods, i.e., 1984-2010, 1984-1990, and 1995-2010. Categories with a single major annual peak were considered unimodal, and those with two peaks as bimodal. For each plankton category, the average seasonal patterns were compared to observe possible overall changes in abundance and phenology between the first and the second periods of the LTER-MC time series. A total of 6 phenological events were determined: **1.** the month of peak abundance (from the average annual cycle), **2.** The onset or start of population increase (day of year), **3.** peak population (day of year), **4.** end of population (day of year) , **5.** duration of phenological season (length in number of days), and **6.** the approximate (month) of peak timing.

2.3.1 *Phenophases*

The cumulative percentile method (Greve, 2005, Mackas et al., 2012) was applied to identify the phenophases, i.e., the dates at which cumulative abundance reached 25, 50 and 75% of the total annual abundance corresponding to the start (onset), middle (peak) and end of phenological season, respectively. The percentage threshold values were chosen to allow comparison with phenological studies in which the same method was applied to LTER-MC zooplankton associations (Mazzocchi *et al.*, 2011) and other plankton datasets (Mackas *et al.*, 2012). Some studies report 15, 50 and 85% as thresholds (Greve *et al.*, 2005). However, setting a start of season day at the point when cumulative abundance is 15% could overestimate the numerical increase of the population and result in earlier estimates of phenology. The cumulative percentile method has been extensively utilized in ecological studies of phenology (Ji *et al.*, 2010) and is well suited to unimodal taxa that are regularly sampled. First, for each taxonomic category, the non-transformed abundances were summed over each year period to obtain the total cumulative abundance for that year.

Then, the percentage contribution of each sampling event to the total annual abundance was calculated. For each year of the time series, the dates of the three phenophases were identified using an integral of the cumulative sum and plotted over the years to show the long-term variability in phenology.

2.3.2. *Duration*

The duration of phenological season was calculated as the time difference between the end and the start of the season as obtained from the cumulative percentile method.

2.3.3. *Timing of central tendency*

The timing of central tendency (Colebrook, 1979, Edwards & Richardson, 2004) was used to estimate the timing of major peak abundance. It was applied to mixed phytoplankton and zooplankton taxa from the CPR plankton time series (Colebrook, 1979, Edwards & Richardson, 2004) and more recently to a Mediterranean zooplankton time series in the Adriatic Sea (Conversi *et al.*, 2009). In the LTER-MC time series, the timing of central tendency was calculated over the whole annual cycle for unimodal taxa; for bimodal taxa, it was calculated separately for the first part of the year (January through June), and the second part of the year (July through December).

General formula:

The timing (T_m) of peak abundance of each month was calculated as:

$$T_{(m)} = \frac{\sum(m \cdot x_m)}{\sum x_m}$$

where:

m= month number 1:12, with 1=Jan, 12=December

x_m= mean abundance in the month m.

Briefly, the sum of mean abundance for each month for each year of the time series was multiplied by the corresponding month number in each year $\sum(m \cdot x_m)$ to obtain a mean sum value. This was divided by a sum of the mean average for each month for each

year $\sum x_m$. For unimodal taxa, the timing was calculated between months 1:12 and for bimodal taxa, the timing was calculated separately for the first half of the year (months 1:6) and second half of the year (months 7:12). The resulting timing is expressed as a decimal month fraction.

Long-term trends in plankton phenology were tested for their significance by applying linear regression analysis, which was applied separately to the two distinct parts of the time series (1984-1990 and 1995-2010). Trends were considered significant * at $0.01 < p < 0.05$, and highly significant ** at $0.001 < p < 0.01$ with a confidence interval set at 95%.

2.4 Correlations with environmental parameters

The possible environmental forcing driving plankton phenology was investigated by considering the local conditions recorded at stn. LTER-MC. The significant changes observed in phenological indexes (of specific plankton taxa) were correlated with the surface (2m) and depth-integrated (0-60 m) anomalies of temperature, salinity, nutrients, and chlorophyll at seasonal and monthly scales. To homogenize the data sets, monthly means of each parameter were calculated to account for variability in sampling frequency. A matrix of years x months was constructed from calculated monthly means. When data were missing, the data was interpolated using the general mean for the month. In the case of chlorophyll, data were missing from January to May in 1995 and were not considered in the general mean.

Anomalies of environmental parameters were calculated based on the general mean of each season or each month in the complete time series (1984-2010), which was in turn calculated from monthly mean values. For each season, the general mean was subtracted from the annual mean and divided by the standard deviation of the general mean. The anomaly value was positive (> 0) when the calculated average was higher than the general mean and negative (< 0) when the anomaly was lower than the general mean.

General formula:

$$N_y = \left(\frac{x_y - x_{ts}}{\sqrt{x_{ts}}}\right)$$

N_y = normalized time series
x_Y = seasonal mean of year y
x_{ts} = general mean of the season in the time series (1984- 2010)
$\sqrt{x_{ts}}$ = standard deviation of the general mean of the season in the time series

The general steps in this analysis are depicted in Fig 2.2. For each taxon, phenological indices were correlated with the appropriate seasonal or monthly environmental anomalies as shown in Step 1. A raw data matrix was created from the selection of specific seasonal or monthly correlates and pair-wise correlation analysis was carried out using the Pearson Product-moment correlation data analysis add-in for Excel. For each respective correlation coefficient (within the appropriate season or month of interest) with a strong negative or positive value, linear regression analysis was applied to determine the significance of the relationship between phenology and the selected environmental correlate. Principal component analysis (PCA) was carried out on a matrix of environmental anomalies and phenology for each taxa with significant long-term changes. Results were visualized and verified using correlograms, linear regressions and bivariate plots and finally with loading and biplots for the results of PCA.

Due to the discrepancy in sampling, phytoplankton and zooplankton datasets were correlated with surface and depth-integrated environmental anomalies accordingly. Phytoplankton concentrations originate from a surface net sample as opposed to the zooplankton sample which is collected as an integrated water column net tow. Thus, phytoplankton phenology was correlated with surface anomalies of temperature and salinity, and zooplankton phenology was correlated with depth integrated anomalies of temperature and chlorophyll at monthly and seasonal scales respectively.

Organization and analysis of raw data files was carried out using Microsoft Excel 2010 with data analysis add-in toolpak for correlation and regression analysis. Input .csv or

.txt files made in excel were created for further analysis in R and JMP. Phenological indexing using the cumulative percentile method was carried out using the software R for statistical analysis (R, 2011) Version 2.15.0 with RStudio Version 0.97.248 (2009-2012) and associated packages for high level plots. Multivariate analysis was carried out using JMP 10.0.2 ©SAS (2012) to identify long-term trends, statistical significance, and correlative relationships between phenology and environmental parameters.

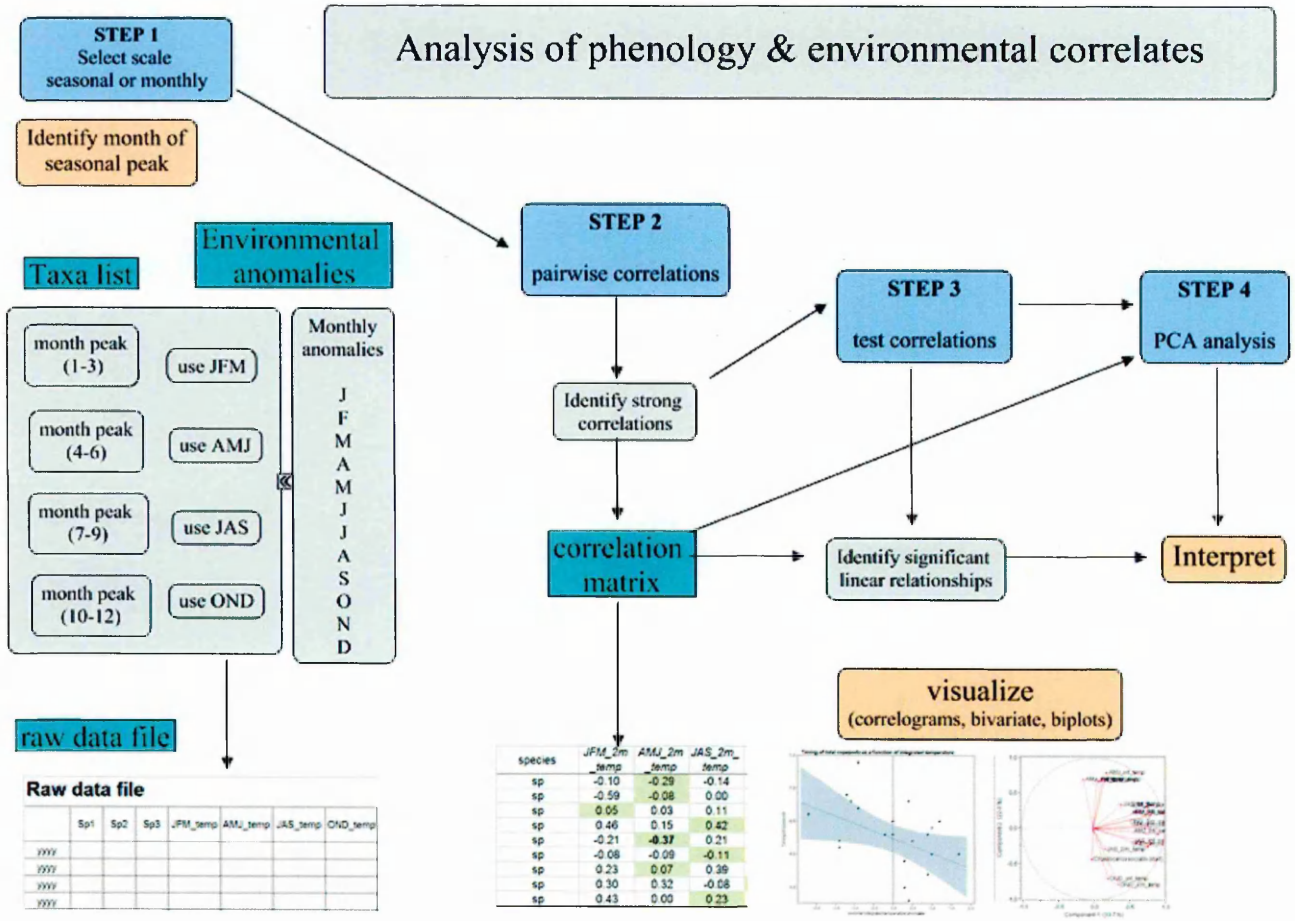

Figure 2.2 Schematic illustration of the steps involved in analysis of environmental drivers on observed changes in phenology.

Chapter 3

LONG-TERM PATTERNS OF PLANKTON PHENOLOGY

3.1 Seasonal patterns of plankton abundance

The abundance of 104 phytoplankton and 88 zooplankton categories was examined in the whole time series (1984-2010) and in the two separate periods 1984-1990 and 1995-2010 to identify the main patterns of seasonal variability and possible changes in the timing and magnitude of annual peaks. For each of these categories, the average annual abundance, the average percent contribution to each respective group, and the long-term trend determined by linear regression are reported in Table 3.1 for phytoplankton and in Table 3.2 for zooplankton.

Table 3.1 Ranked average annual abundance and standard deviation (cells ml^{-1}), and average contribution (%) of selected phytoplankton categories to total diatoms, dinoflagellates, coccolithophores, and other flagellates. Average contribution is calculated as an average of annual percent contribution over 23 year period (1984-2010). Phytoplankton categories with significant trends in long-term abundance are indicated as positive or negative with corresponding directional arrows as up or down in red and blue respectively. Significance (p-value) is based on linear trend analysis with significance level at 95% C.I.

	Average annual abundance (cells ml^{-1})	SD (cells ml^{-1})	Average contribution (%)	1984-1990 trend		1995-2010 trend	
				Trend	p-value	Trend	p-value
Total phytoplankton	9435.71	3955.654		↓	0.0320	–	–
Total diatoms			52.86	↓	0.0084	–	–
Total other flagellates			42.27	–	–	–	–
Total dinoflagellates			2.67	–	–	–	–
Total coccolithophores			2.20	–	–	–	–
diatoms							
Chaetoceros spp.	864.84	1071.19	15.57	–	–	–	–
Chaetoceros tenuissimus	797.18	444.31	15.46	↓	0.0326	–	–
Skeletonema pseudocostatum	762.26	610.22	14.92	–	–	–	–
Leptocylindrus danicus	569.54	312.02	13.39	–	–	–	–
Chaetoceros socialis	286.17	395.56	5.05	↓	0.0227	–	–
Thalassiosira spp.	175.62	172.19	3.86	↓	0.0194	–	–
Pseudo-nitzschia delicatissima	115.79	74.06	2.71	–	–	–	–
Thalassiosira cf. allenii	112.02	121.65	2.50	–	–	–	–
Skeletonema menzelii	138.44	191.28	2.27	–	–	↑	0.0408
Cyclotella atomus var. *gracilis*	83.13	112.86	2.27	–	–	–	–
Pseudo-nitzschia galaxiae	117.11	121.41	2.16	–	–	↑	0.0193
Cylindrotheca closterium	119.40	189.95	2.11	↓	0.0099	–	–
Bacteriastrum parallelum	131.39	252.53	1.98	–	–	–	–
Minidiscus comicus	84.46	149.41	1.76	–	–	–	–
Chaetoceros throndsenii	47.46	97.07	1.23	–	–	–	–
Pseudo-nitzschia pseudodelicatissima	33.36	40.05	0.79	–	–	–	–
Cerataulina pelagica	26.91	18.17	0.75	–	–	–	–
Chaetoceros curvisetus	24.98	22.52	0.71	↓	0.0129	↓	0.0129
Chaetoceros "curvi-curvi"	5.37	5.92	0.65	–	–	–	–
Cyclotella atomus var. *atomus*	36.42	99.70	0.58	–	–	–	–
Minutocellus polymorphus	28.07	86.21	0.49	–	–	–	–
Thalassiosira mediterranea	16.81	39.54	0.46	–	–	↓	0.0272
Chaetoceros simplex	24.12	31.73	0.44	–	–	↑	0.0063
Leptocylindrus minimus	20.73	22.97	0.44	–	–	–	–
Centric diatoms < 10 µm	21.62	52.93	0.32	–	–	–	–
Chaetoceros minimus	16.90	41.31	0.31	–	–	–	–
Chaetoceros contortus	35.03	35.72	0.29	–	–	–	–
Thalassionema nitzschioides	11.15	9.83	0.26	–	–	–	–
Dactyliosolen fragilissimus	10.54	14.18	0.24	–	–	–	–
Asterionellopsis glacialis	11.22	17.40	0.22	–	–	–	–
Pseudo-nitzschia galaxiae "small morphotype"	10.75	11.28	0.22	–	–	–	–
Thalassiosira rotula	9.66	17.66	0.21	–	–	–	–
Pseudo-nitzschia spp.	9.80	14.89	0.19	–	–	–	–
Chaetoceros diadema	9.91	11.57	0.19	–	–	–	–

Table 3.1 (cont).

phytoplankton	Average annual abundance (cells ml^{-1})	SD (cells ml^{-1})	Average contribution (%)	1984-1990 trend		1995-2010 trend	
				Trend	p-value	Trend	p-value
Chaetoceros affinis	1.89	2.45	0.12	–	–	↑	0.0452
Cyclotella spp.	4.45	5.88	0.12	–	–	–	–
Lithodesmium cf. *variabile*	3.22	6.45	0.11	–	–	–	–
Bacteriastrum spp.	4.35	12.94	0.10	–	–	–	–
Chaetoceros protuberans	3.53	4.25	0.08	↓	0.0247	–	–
Dactyliosolen phuketensis	2.52	3.58	0.08	–	–	–	–
Pseudo-nitzschia multistriata	5.18	7.91	0.08	–	–	↑	0.0097
Bacteriastrum furcatum	4.76	15.65	0.08	–	–	–	–
Chaetoceros pseudocurvisetus	3.26	3.64	0.08	–	–	–	–
Dactyliosolen blavyanus	3.86	3.54	0.07	–	–	–	–
Pennate diatoms > 10 µm	2.33	2.06	0.06	–	–	–	–
Chaetoceros anastomosans	16.23	22.53	0.05	–	–	–	–
Lauderia annulata	1.86	1.89	0.05	–	–	–	–
Thalassionema bacillare/frauenfeldii	1.92	2.22	0.05	↓	0.0458	–	–
Proboscia alata	1.78	2.36	0.04	–	–	–	–
Guinardia striata	1.51	1.33	0.04	–	–	–	–
Pennate diatoms < 10 µm	1.98	3.42	0.04	–	–	–	–
Centric diatoms > 10 µm	1.34	2.00	0.03	–	–	–	–
Chaetoceros lorenzianus	0.53	0.62	0.01	–	–	–	–
Leptocylindrus mediterraneus	0.44	0.79	0.01	–	–	↑	0.0104
Chaetoceros peruvianus	0.34	0.35	0.01	–	–	–	–
dinoflagellates							
Naked dinoflagellates < 15 µm	116.39	33.04	0.61	–	–	–	–
Naked dinoflagellates > 15 µm	20.79	17.05	0.10	–	–	–	–
Thecate dinoflagellates < 15 µm	19.06	10.83	0.09	–	–	–	–
Prorocentrum triestinum	10.53	13.17	0.05	–	–	↓	0.0402
Calciodinelloideae n.d.	5.78	4.99	0.03	–	–	–	–
Thecate dinoflagellates > 15 µm	3.58	3.94	0.02	↓	0.0056	↓	0.0483
Heterocapsa niei	2.61	2.85	0.01	–	–	–	–
Lessardia elongata	2.05	1.26	0.01	–	–	–	–
Protoperidinium spp.	1.77	1.49	0.01	–	–	↑	0.0136
Oxytoxum variabile	1.26	0.98	0.01	↑	0.0048	–	–
Protoperidinium bipes	0.91	1.04	0.00	–	–	–	–

Table 3.1 (cont).

phytoplankton	Average annual abundance (cells ml^{-1})	SD (cells ml^{-1})	Average contribution (%)	1984-1990 trend		1995-2010 trend	
				Trend	p-value	Trend	p-value
coccolithophores							
Emiliana huxleyi	109.34	104.03	54.27	–	–	–	–
Undetermined coccolithophores	45.15	14.46	29.62	–	–	–	–
Calciopappus caudatus	8.16	6.46	4.97	–	–	–	–
Syracosphaera pulchra	4.19	3.33	2.77	–	–	–	–
Syracosphaera molischii	1.13	1.24	0.91	–	–	–	–
Calciosolenia brasiliensis	1.25	0.97	0.88	–	–	–	–
Rhabdosphaera clavigera	0.74	0.65	0.54	–	–	–	–
Acanthoica quattrospina	0.62	0.68	0.42	–	–	–	–
Ophiaster spp.	0.54	0.59	0.36	–	–	–	–
Algyrosphaera robusta	0.17	0.23	0.13	–	–	–	–
Coronosphaera mediterranea	0.08	0.09	0.07	–	–	–	–
other flagellates							
Undetermined phytoflagellates < 10 µm	3472.44	1655.58	85.88	–	–	–	–
Undetermined cryptophyceans < 10 µm	158.91	91.78	4.07	↑	0.0002	–	–
Ollicola vangoorii	48.22	18.18	1.44	–	–	–	–
Pyramimonas spp.	32.59	28.70	1.01	–	–	↓	0.0427
Heterotroph flagellates	30.42	43.46	0.58	–	–	↑	<0.0001
Eutreptiella spp.	17.76	69.12	0.54	–	–	–	–
Pseudoscourfieldia marina	26.02	23.08	0.51	–	–	–	–
Paulinella ovalis	14.24	9.19	0.35	–	–	–	–
Phaeocystis spp.	14.15	18.01	0.34	–	–	↓	<0.0001
Dinobryon faculiferum	10.54	5.73	0.34	–	–	–	–
Leucocryptos marina	15.10	15.39	0.30	–	–	↑	<0.0001
Undetermined phytoflagellates > 10 µm	8.90	6.87	0.29	–	–	–	–
Dinobryon coalescens	9.04	16.71	0.28	–	–	–	–
Undetermined cryptophyceans > 10 µm	3.20	4.16	0.14	–	–	–	–
Tetraselmis spp.	4.91	4.49	0.13	–	–	–	–
Chrysochromulina spp.	4.99	6.32	0.11	–	–	↓	0.0355
Pachysphaera spp.	3.43	2.33	0.09	–	–	–	–
Apedinella spinifera	1.91	1.41	0.05	↓	0.0500	–	–
Diplostauron cf. *elegans*	2.63	3.49	0.05	–	–	↑	0.0229
Meringosphaera mediterranea	2.00	2.28	0.04	–	–	–	–
Rhizomonas setigera	1.83	2.38	0.03	–	–	↑	0.0137
Dictyocha fibula	0.28	0.18	0.01	–	–	–	

Table 3.2 Ranked average annual abundance and standard deviation (Ind. m^{-3}), and average contribution (%) of groups to total zooplankton and of selected (≥ 0.01%) copepod and cladoceran taxa to the total of respective groups. Average contribution is calculated as an average of annual percent contribution over 23 year period (1984-2010). Zooplankton categories with significant trends in long-term abundance are indicated as positive or negative with corresponding directional arrows as up or down in red and blue respectively. Significance (p-value) is based on linear trend analysis with significance level at 95% C.I.

zooplankton	Average annual abundance (Ind.m^{-3})	SD (Ind.m^{-3})	Average contribution (%)	1984-1990 trend		1995-2010 trend	
				Trend	p-value	Trend	p-value
Total zooplankton	1688.41	355.31		–	–	↑	0.0378
Total copepods	1072.21	242.43	63.89	–	–	–	–
Total cladocerans	354.11	190.34	20.29	–	–	–	–
Appendicularians	157.56	43.36	9.46	–	–	↑	0.0129
Decapod larvae	27.87	19.43	1.78	↓	0.0111	–	–
Doliolids	24.11	19.71	1.43	–	–	–	–
Cirriped larvae	13.02	6.59	0.79	↓	0.0025	–	–
Gastropods (sum of Gastropod larvae + Pteropods)	12.06	4.61	0.75	–	–	↑	0.0062
Chaetognaths	8.35	5.52	0.49	↓	0.043	↑	0.0004
Hydromedusae	4.06	1.85	0.25	–	–	–	–
Polychaete larvae	3.12	0.76	0.19	↓	0.0451	–	–
Bivalve larvae	3.02	2.59	0.17	–	–	–	–
Salps	2.79	4.36	0.15	–	–	–	–
Siphonophores	1.81	0.85	0.11	–	–	–	–
Ostracods	1.65	0.90	0.10	–	–	–	–
Echinoderm larvae	1.59	1.66	0.09	–	–	–	–
Pisces larvae + eggs	0.92	0.41	0.06	↓	0.0073	–	–
Ascidiacean larvae	0.16	0.19	0.01	–	–	–	–
Amphipods	0.04	0.04	<0.01	–	–	↑	0.0003
Isopod Epicaridean larvae	0.04	0.05	<0.01	–	–	–	–
Copepods							
Calanoid juveniles (juv of *Clausocalanus* spp.+*P.parvus*)	304.48	99.03	28.18	–	–	–	–
Acartia clausi	157.25	74.46	14.51	–	–	–	–
Paracalanus parvus (only adults)	116.77	67.41	10.53	–	–	–	–
Centropages typicus	89.18	46.28	8.05	–	–	–	–
Temora stylifera	70.76	31.15	6.78	–	–	–	–
Oithona spp. males+juv.	60.95	19.11	5.89	–	–	–	–
Oncaeidae	39.10	12.49	3.74	–	–	–	–
Calocalanus spp.	33.65	11.24	3.29	↑	0.0376	↑	0.0171
Clausocalanus furcatus females	19.41	10.85	1.79	–	–	–	–
Ctenocalanus vanus	18.04	9.36	1.77	–	–	↑	0.005
Clausocalanus spp. males	18.48	9.28	1.67	↓	0.0125	–	–
Oithona similis females	16.58	9.82	1.54	↓	0.0289	–	–
Clausocalanus paululus females	13.79	4.52	1.30	↓	0.0232	–	–
Farranula rostrata	12.78	4.21	1.24	–	–	↑	0.0393
Paracalanus nanus	12.73	4.47	1.24	–	–	–	–
Corycaeus spp.	11.24	1.85	1.09	–	–	–	–
Euterpina acutifrons	11.13	5.50	1.08	–	–	↑	0.0156
Oithona nana females	10.62	9.63	1.08	↑	0.0039	–	–
Clausocalanus arcuicornis females	10.06	4.43	1.00	–	–	↓	0.0088
Clausocalanus pergens females	9.18	9.17	0.80	↓	0.0004	–	–
Oithona plumifera females	6.04	3.07	0.57	–	–	–	–
Oithona longispina females	3.91	2.18	0.36	–	–	–	–

Table 3.2 (cont).

zooplankton	Average annual	SD (Ind.m^{-3})	Average contribution	1984-1990 trend		1995-2010 trend	
				Trend	p-value	Trend	p-value
Isias clavipes	3.08	2.43	0.30	↑	0.0003	↓	0.0034
Candacia spp.	2.45	0.87	0.23	–	–	–	–
Paracalanus denudatus	2.08	2.84	0.20	↑	<0.0001	↓	0.0001
Nannocalanus minor	2.08	1.58	0.20	↓	0.0010	↑	<0.0001
Calanidae juveniles (unid.)	1.73	1.72	0.17	–	–	↓	0.0016
Pleuromamma spp.	1.52	0.82	0.15	–	–	↑	0.0382
Centropages ponticus	1.54	1.70	0.14	–	–	↓	0.0143
Clausocalanus lividus females	1.45	0.95	0.14	–	–	–	–
Clausocalanus jobei females	1.09	0.77	0.11	–	–	↓	0.0417
Acartia negligens	0.85	1.36	0.09	–	–	↑	<0.0001
Oithona setigera females	0.79	0.76	0.08	↑	<0.0001	↓	0.0019
Mecynocera clausi	0.65	0.60	0.06	–	–	–	–
Centropages kroyeri	0.41	0.64	0.05	–	–	↓	0.0123
Clausocalanus mastigophorus females	0.42	0.40	0.04	↑	0.0041	↓	<0.0001
Neocalanus gracilis	0.40	0.24	0.04	–	–	↑	0.0007
Lucicutia spp.	0.43	0.32	0.04	–	–	–	–
Centropages violaceus	0.41	0.23	0.04			–	–
Clausocalanus parapergens females	0.42	0.53	0.04	↓	<0.0001	↓	0.0003
Diaixis spp.	0.37	0.48	0.03	–	–	–	–
Mesocalanus tenuicornis	0.34	0.55	0.03	–	–	↑	<0.0001
Acartia margalefi	0.32	0.55	0.03	↓	0.0094	–	–
Calanus helgolandicus	0.33	0.72	0.03	–	–	–	–
Sapphirina spp.	0.28	0.28	0.03	–	–	↑	0.0038
Acartia discaudata	0.28	0.45	0.03	–	–	–	–
Oithona atlantica females	0.30	0.53	0.02	↓	<0.0001	–	–
Euchaeta spp.	0.23	0.22	0.02	↑	0.0003	–	–
Scolecithricella spp.	0.20	0.14	0.02	↑	0.0381	–	–
Harpacticoida unid.	0.16	0.08	0.02	↑	0.0496	–	–
Oithona decipiens females	0.16	0.28	0.01	↑	0.0009	–	–
Pontellidae	0.15	0.09	0.01	–	–	–	–
Microsetella spp.	0.12	0.18	0.01	–	–	–	–
Paracartia grani	0.11	0.18	0.01	–	–	–	–
Heterorhabdus papilliger	0.10	0.06	0.01	–	–	–	–
Eucalanidae	0.11	0.13	0.01	↓	0.0424	–	–
Clytemnestra rostrata	0.09	0.08	0.01	↓	0.0066	–	–
Oithona tenuis females	0.08	0.13	0.01	–	–	–	–
Scolecithrix spp.	0.07	0.07	0.01	–	–	↓	0.0016
Scaphocalanus spp.	0.06	0.06	0.01	–		–	–
Copilia spp.	0.05	0.15	0.01	–	–	–	–
Acartia danae	0.04	0.05	<0.01	–	–	–	–
Haloptilus spp.	0.03	0.03	<0.01	–	–	–	–
Macrosetella gracilis	0.02	0.03	<0.01	–	–	↑	0.0014
Pontellina plumata	0.02	0.03	<0.01	–	–	–	–
Cladocerans							
Penilia avirostris	243.65	136.08	68.29	–	–	–	–
Evadne spinifera + *Pseudoevadne tergestina*	96.26	62.09	27.09	–	–	–	–
Podon intermedius + *Pleopis polyphemoides*	14.20	19.30	4.61	–	–	–	–

3.1.1 Phytoplankton seasonal cycle

The total phytoplankton assemblage was shaped by the contribution of four dominant groups, i.e., diatoms, other flagellates, dinoflagellates and coccolithophores. Diatoms accounted for 53% of total cell concentration on an average annual base, followed by other flagellates (42%), dinoflagellates (3%) and coccolithophores (2%). More than 50% of total annual phytoplankton abundance occurred between April and June (Fig. 3.1).

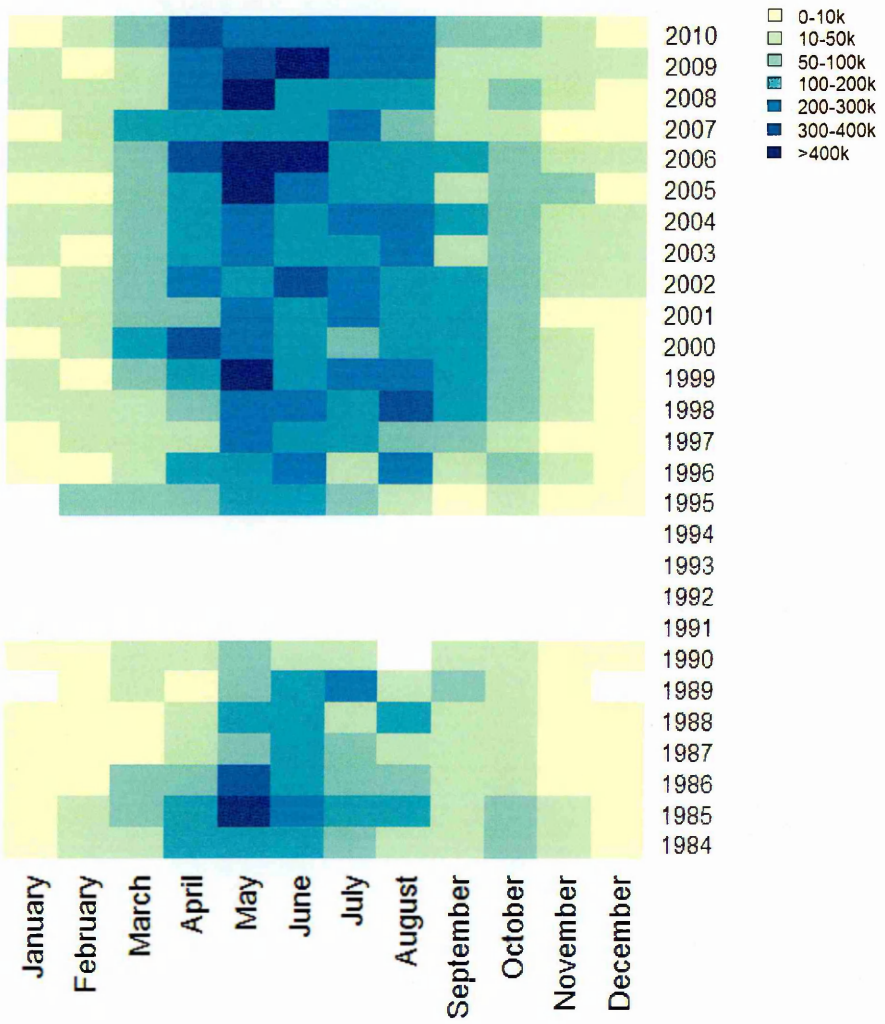

Figure 3.1 Long-term seasonal abundance of total phytoplankton (cells ml^{-1}). Heatmap colors range from pale shades reflecting lowest concentrations of 0-1000 ($k=10^3$) to darker intensity with a maximum of > 400,000 cells ml^{-1}. White indicates months and years of missing data.

The general annual pattern of total phytoplankton in 1984-2010 showed the lowest concentrations in January-February, an increase in March, a major peak in May (2.6 stdev $1.6x10^4$ cells ml^{-1}) and then a regular decline interrupted by a secondary peak in August before reaching again the annual low in November-December (7.2 stdev $4.9x10^4$ cells ml^{-1}) (Fig. 3.2 A). Total phytoplankton abundance was higher in the second part of the time series and noticeable during the two peak periods (May and August). The absence of the secondary August peak in the first part of the time series highlighted its appearance and establishment as a regular feature of phytoplankton dynamics only starting from the late '90s. The most abundant phytoplankton groups, i.e., diatoms, other flagellates and dinoflagellates had a steep increase in spring and a major peak in May-June, (Fig. 3.2 B,C,D).

Diatoms and other flagellates were more abundant in the second part of the time-series. Diatoms acquired a secondary peak in August which was observed in the total phytoplankton (Fig. 3.2B). The mean abundance of other flagellates in May nearly doubled from a peak of $7.3x10^3$ to $12.1x10^3$ cells ml^{-1} from the first to the second part of the time series (Fig. 3.2C). The opposite change was observed in dinoflagellates; in the second part of the time-series their peak in May-June decreased and the autumn one disappeared (Fig 3.2D). Coccolithophores were the less abundant group and showed the most remarkable differences between the two parts of the time-series (Fig. 3.2E); in 1984-1990, the group had two major peaks in June and August, while in 1995-2010 a single peak appeared later in October, after a smooth increase of spring concentrations which were maintained through summer.

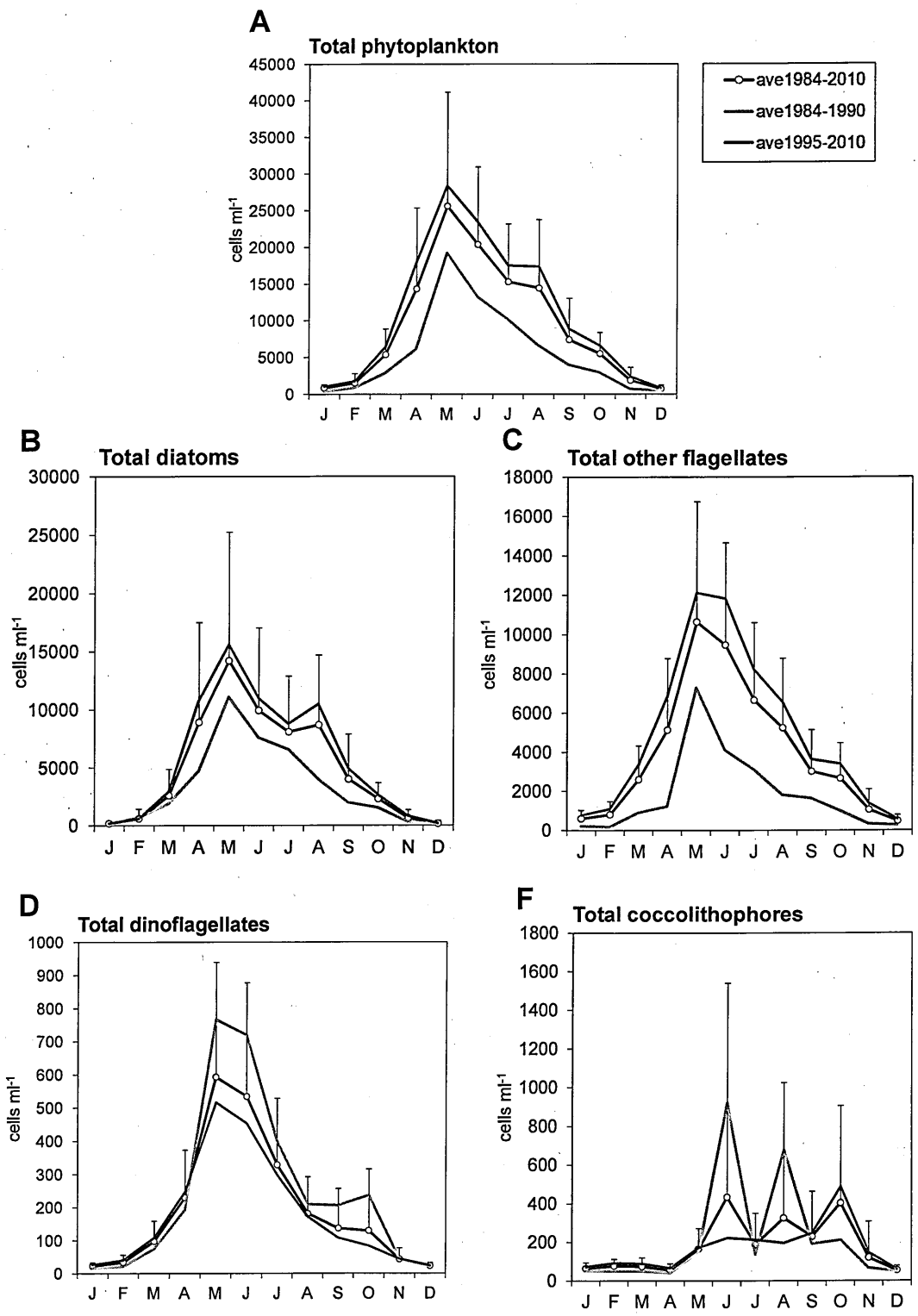

Figure 3.2 Average seasonal cycle of (A) total phytoplankton and groups ranked in order of their contribution to total phytoplankton abundance; (B) diatoms (C) dinoflagellates , (D) coccolithophores, and (F) other flagellates. Three periods are depicted:the whole time series of 23 years (1984-2010) in black with standard deviation bars; part I spanning 7 years (1984-1990) in blue, and part II spanning 16 years (1995-2010) in red.

3.1.2 Changes in the timing of seasonal peaks

Among diatoms, several abundant species showed an earlier timing of seasonal peaks and changes in abundance between the first and second part of the time series (Fig. 3.3). *Chaetoceros* spp. shifted the month and magnitude of peak abundance from June to May. The abundance increased from a maximum of $1.7x10^3$ to $3.7x10^3$ cells ml^{-1} in June (part I) and May (part II) respectively.The peak month of *Skeletonema pseudocostatum* switched from July to May after 1995 with a notable increase of abundance in the second part of the time series. This taxon also displayed high variability as indicated by the wide range of standard deviation (Fig. 3.3B). *Minidiscus comicus* anticipated the peak from September to August (Fig. 3.3C). *Thalassiosira* cf. *allenii* changed remarkably, with a displacement of the highest abundances from May-July in the first part of the time series to March-May in the second part (Fig. 3.3D). The *Pseudo-nitzschia delicatissima* group comprised of several species also showed a distinctly earlier spring peak that shifted from May to April and a higher but not anticipated summer peak in September (Fig. 3.3E). *Chaetoceros "curvi-curvi"* changed the month of peak abundance from May to April (Fig. 3.3F).

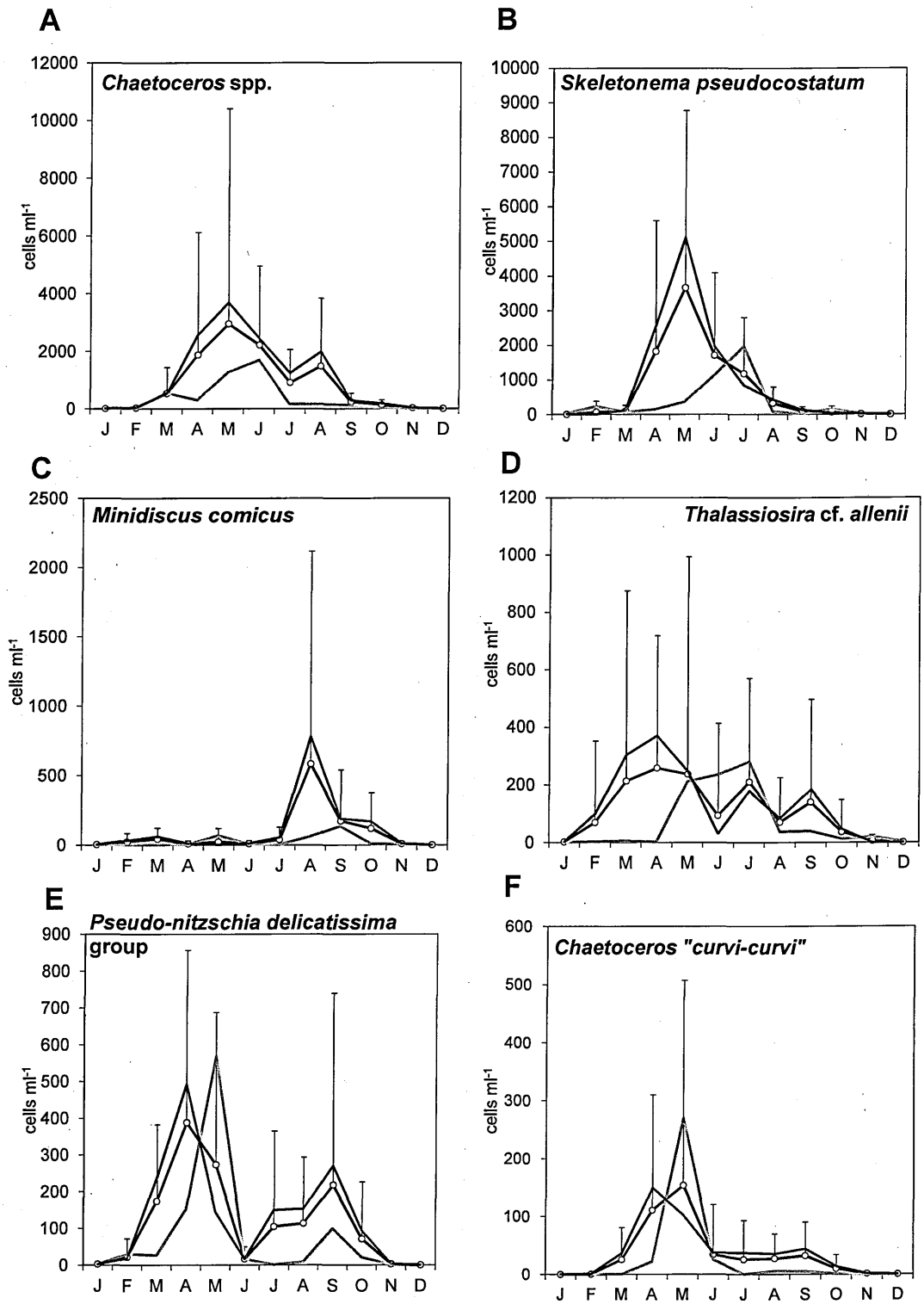

Figure 3.3 Average seasonal cycles of diatom species with an earlier peak of abundance in the second part of the time series. Whole time series (1984-2010) in black) with bars of the standard deviation, first period (1984-1990) in blue, and second period (1995-2010) in red.

Conversely, other abundant diatom taxa showed a delayed seasonal peak in the second part of the time series (Fig 3.4). *Chaetoceros tenuissimus* shifted from May to June and showed a second peak in August that was not present before 1995 (Fig. 3.4 A). The average abundance between April and June did not change, however the secondary peak abundance in August increased from 435.6 to 2084.6 cells ml^{-1} between the two time periods. There was a significant negative (p=0.0326) trend in abundance from 1984 to 1990 (n=7) (Table 3.1). The peak abundance of *C. throndsenii* also occurred one month later in June and decreased after 1995 (Fig. 3.4 B). *Pseudo-nitzschia pseudodelicatissima* shifted the month of peak abundance from May to June, still maintaining similar concentrations (Fig. 3.4 C). *Cerataulina pelagica* shifted the seasonal peak from June to July after 1995 (Fig. 3.4 D).

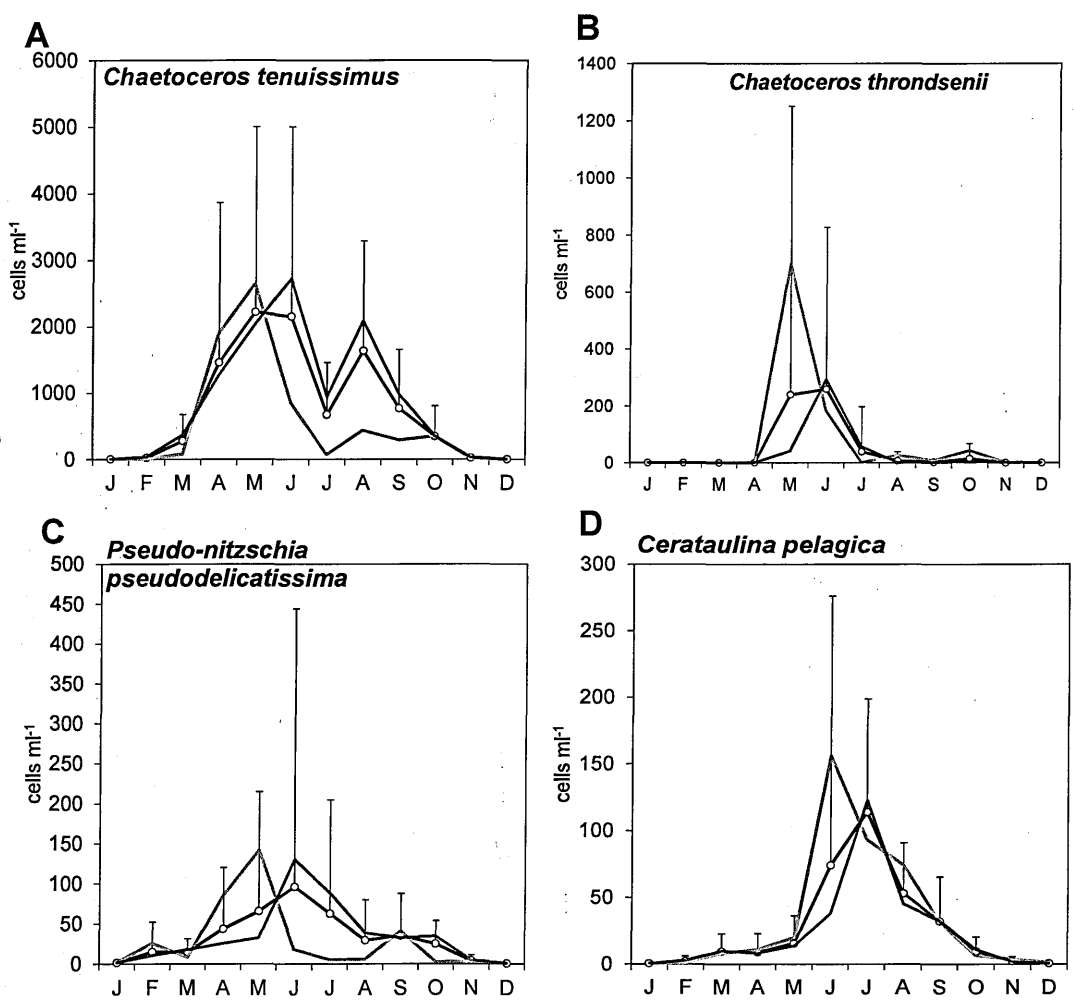

Figure 3.4 Average seasonal cycles of diatom with a delayed peak of abundance in the second part of the time series. Whole time series (1984-2010) in black) with bars of the standard deviation, first period (1984-1990) in blue, and second period (1995-2010) in red.

Within other flagellates, the abundant undetermined cryptophyceans <10μm shaped the overall pattern of total abundance of the group (Fig. 3.2 C). This category shifted the annual peak from July to May and increased in abundance in October (Fig. 3.5A); it had a positive, significant ($p=0.0002$) trend from 1984 to 1990 ($n=7$) (Table 3.1). The seasonal peak of *Ollicola vangoorii* anticipated from May to April in the second part of the time series (Fig. 3.5B). The peak abundance occurred earlier and at greater magnitude in part II for the flagellate *Leucocryptos marina* (Fig. 3.5C). *Dinobryon faculiferum* changed the range of seasonal peak abundance from two distinct periods in May and August to a smoother peak from May to June. In this case, the secondary peak in August was anticipated and dampened to a lower abundance in June (Fig. 3.5D).

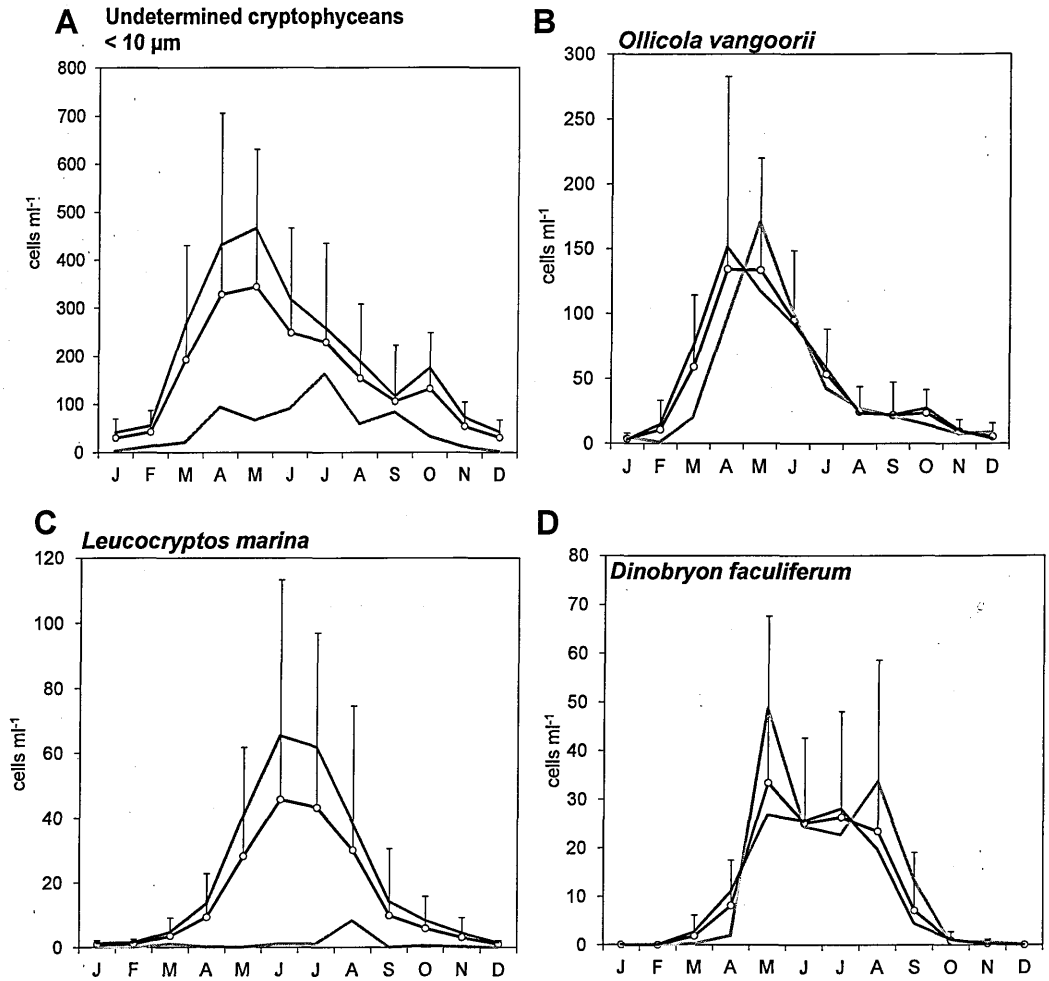

Figure 3.5 Species of the category other flagellates which showed a change in the timing of seasonal peak and in abundance. Whole time series (1984-2010) in black) with bars of the standard deviation, first period (1984-1990) in blue, and second period (1995-2010) in red.

Within dinoflagellates, the size class of >15μm naked dinoflagellates showed a reduction in the magnitude of peak abundance and a change from bimodal peaks in June and October to a single peak in May (Fig. 3.6A). *Prorocentrum triestinum* shifted the primary month of peak abundance from May to a much lower peak in April (Fig. 3.6B)

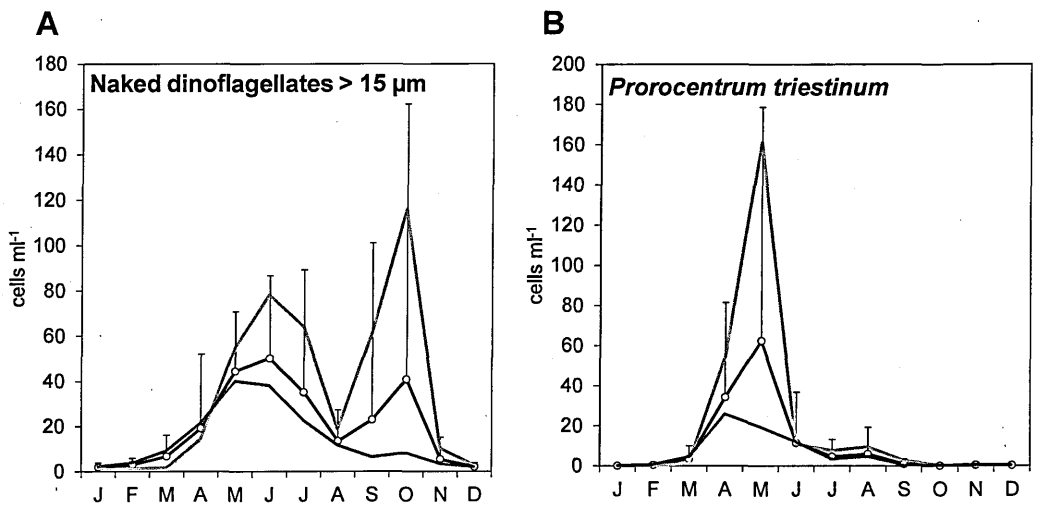

Figure 3.6 Naked dinoflagellates >15μm (A), and *Prorocentrum triestinum* (B) which showed a change in seasonal peak timing between two periods of the time series. Whole time series (1984-2010) in black) with bars of the standard deviation, first period (1984-1990) in blue, and second period (1995-2010) in red.

Within coccolithophores, the dominant species *Emiliania huxleyi* shaped the seasonal pattern of the group (Fig.3.2D). This species shifted from a distinct bimodal pattern of abundance with peaks in June and August to a single peak in October (Fig. 3.7A). The less abundant species *Syracosphaera pulchra* had a bimodal pattern in both parts of the time series but showed anticipated peaks from May and September to March and in the second part. Overall, within coccolithophores, there were no significant long-term trends in abundance.

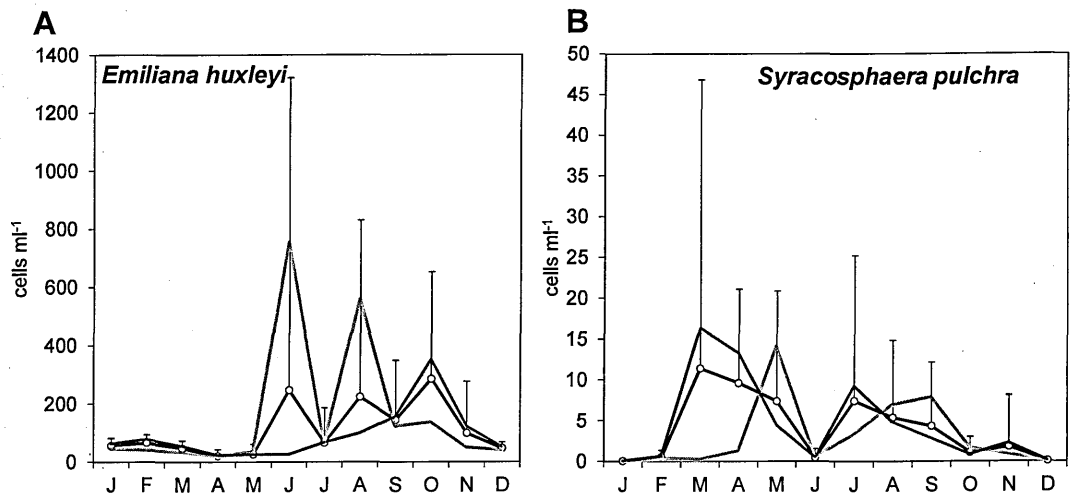

Figure 3.7 Coccolithophores *Emiliania huxleyi* (A), and *Syracosphaera pulchra* (B), which showed a change in timing of seasonal peak between two periods of the time series. Whole time series (1984-2010) in black) with bars of the standard deviation, first period (1984-1990) in blue, and second period (1995-2010) in red.

3.2. Zooplankton seasonal cycle

The seasonal cycles of the most abundant groups, which accounted together for 95.4% of total zooplankton abundance, are reported in Fig.3.8. The average annual cycle of total zooplankton was characterized by minimum abundance in December-January, a first minor peak in April and a primary peak in August (Fig 3.8A). There was a significant ($p=0.0378$) positive trend in abundance after 1995 ($n=16$) (Table 3.2) but the general seasonal pattern was similar between both periods of the time series, with only slightly higher abundances in the first period in correspondence of the peaks (Fig. 3.8A). Total copepods, which comprised 64% of total zooplankton community showed changes in their seasonal cycle (Fig. 3.8C). The two high and sharp peaks recorded in April and July-August in 1984 -1990 were replaced in 1995-2010 by a smoother and more prolonged spring peak (April-June) and lower summer abundances in August-September. Cladocerans contributed to zooplankton only in summer; they reached the peak abundance in August ($1.94x10^3$ stdev $9.9x10^2$ $Ind.m^{-3}$) (Fig. 3.8B) and accounted for 52% of total abundance. Appendicularians contributed 9.46% to total zooplankton abundance and had two major peaks of highest abundances in February-March and in August-October (Fig. 3.8D).The patterns of abundance in the two parts of the time series overlapped until April and diverged afterwards, when higher abundances occurred in the second part. The abundance of this group increased significantly ($p=0.0129$) in the second part of the time series (Table 3.2). Decapod larvae, which represented the most abundant meroplanktonic group, showed in 1995-2010 a major peak in May-June, in addition to the peak in March that occurred similarly in both parts of the time series (Fig.3.8E). This group had a significant ($p=0.0111$) negative trend in abundance in the first period ($n=7$) (Table 3.2).

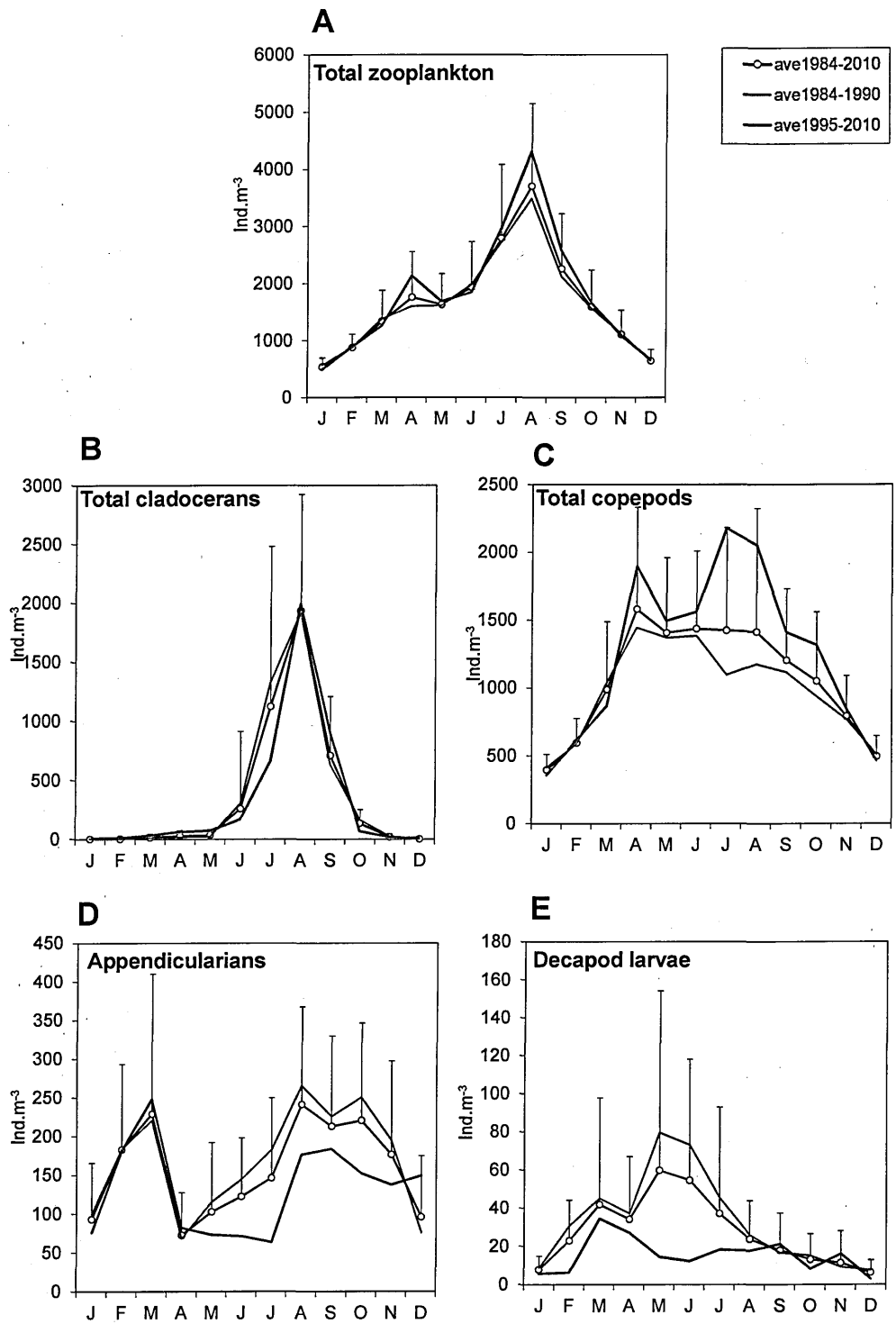

Figure 3.8 Average seasonal cycle of abundance for (A) total zooplankton, (B) total cladocerans, (C) total copepods, (D) appendicularians, and (E) decapod larvae. Three periods are depicted: the whole time series of 23 years (1984-2010) in black with standard deviation bars; part I spanning 7 years (1984-1990) in blue, and part II spanning 16 years (1995-2010) in red.

3.2.1 Changes in the timing of seasonal cycles

Chaetognaths and gastropods (larvae + pteropods) showed a significantly positive trend in abundance in the second period of the time series (Table 3.2). Chaetognaths had a relatively low abundance throughout the year (< 5 Ind.m^{-3}), which increased steadily from July until a peak abundance of 21.00 ±14.5 Ind.m^{-3} in October (Fig. 3.9A). The long-term abundance was much higher in the second part of the time series and showed a more pronounced peak than in the first part. In contrast, gastropods had a bimodal cycle with a peak in spring and secondary peak in summer due to the contribution of pteropods which comprised the major abundance in August (Fig. 3.9B). The April peak of gastropods shifted to February in the second period of the time series, while the secondary peak in abundance declined but remained in August. The peak declined from an average of 29.10 to 20.00 Ind.m^{-3} from the first to the second part of the time series. Although the magnitude of peak abundance decreased, the overall abundance had a significant positive trend (p=0.0062, n=190) in the second period (Table 3.2).

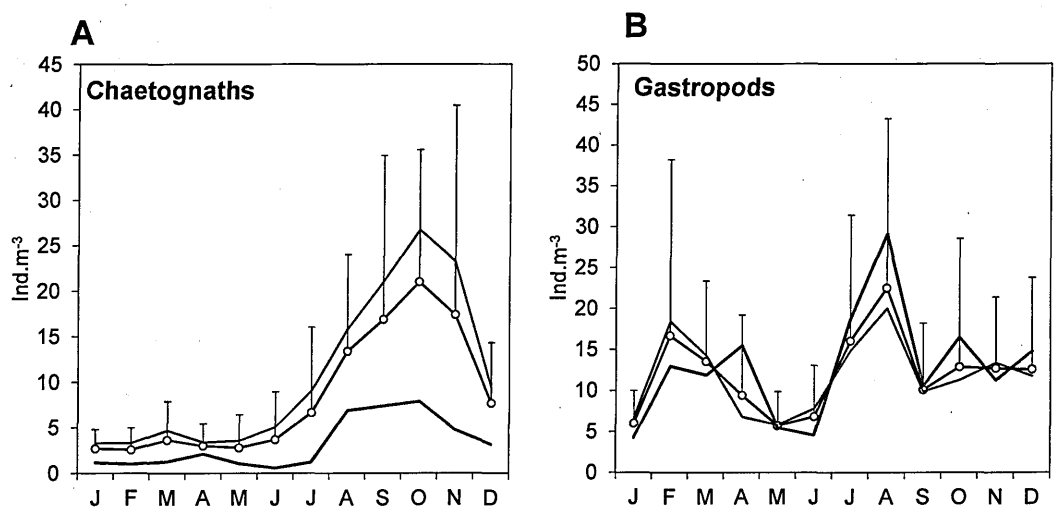

Figure 3.9 Average seasonal cycles of zooplanktonic groups which showed significant positive trends in abundance in the second period of the time series: (A) chaetognaths, and (B) gastropods (sum of Gastropod larvae and Pteropods). Whole time series (1984-2010) in black) with bars of the standard deviation, first period (1984-1990) in blue, and second period (1995-2010) in red.

Some groups with a peak in the first part of the year declined in abundance after 1995; this is the case of three meroplanktonic groups (cirriped larvae, bivalve larvae, echinoderm larvae) and Hydromedusae (Table 3.2) Cirriped larvae showed a decline in the highest peak in February, while the reverse occurred in November, when a small secondary peak increased in magnitude after 1995 (Fig. 3.10A). This group presented a significant negative trend in abundance in both parts of the time series (Table 3.2). Bivalve larvae had a peak abundance in April in both parts of the time series but its extension was lower in 1995-2010 (Fig.3.10B). Hydromedusae showed a variable pattern of abundance with two peaks in the first period of the time series and three distinct peaks in the second period (Fig. 3.10C). Echinoderm larvae, while also low in abundance (an average of < 14 Ind.m^{-3}), showed a much lower peak in March in the second part of the time series (Fig.3.10D).

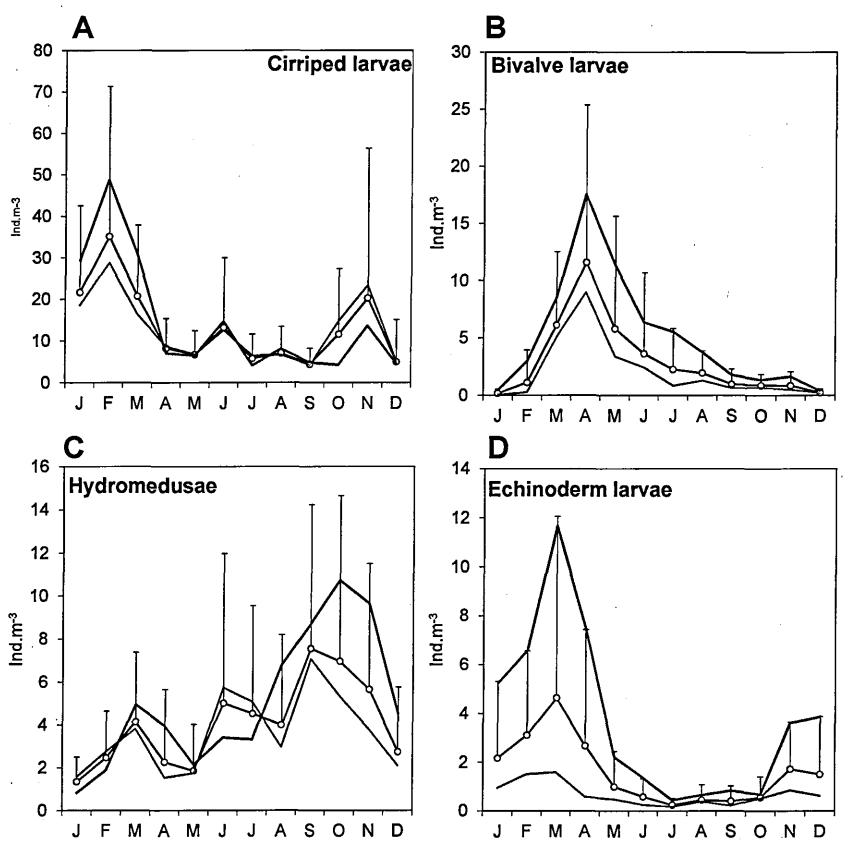

Figure 3.10 Average seasonal cycles of zooplanktonic groups that decreased in abundance after 1995: (A) cirriped larvae, (B) bivalve larvae, (C) hydromedusae, and (E) echinoderm larvae. Whole time series (1984-2010) in black) with bars of the standard deviation, first period (1984-1990) in blue, and second period (1995-2010) in red.

Within copepods, some of the most abundant species showed a decline in abundance and corresponding change in the month of peak occurrence. Among calanoid copepods, *Acartia clausi* anticipated the peak from July to June (Fig. 3.11A); *Centropages typicus* changed seasonality from a bimodal distribution with separated peaks in April and July to a single peak in May-June (Fig. 3.11B), *Paracalanus parvus* adults maintained the same seasonal timing with a peak in August, but the magnitude of abundance decreased by nearly half the abundance from 566.2 to 253.3 ind.m^{-3} during summer (Fig.3.11C); *Temora stylifera* had a lower and earlier peak in September (Fig.3.11D).

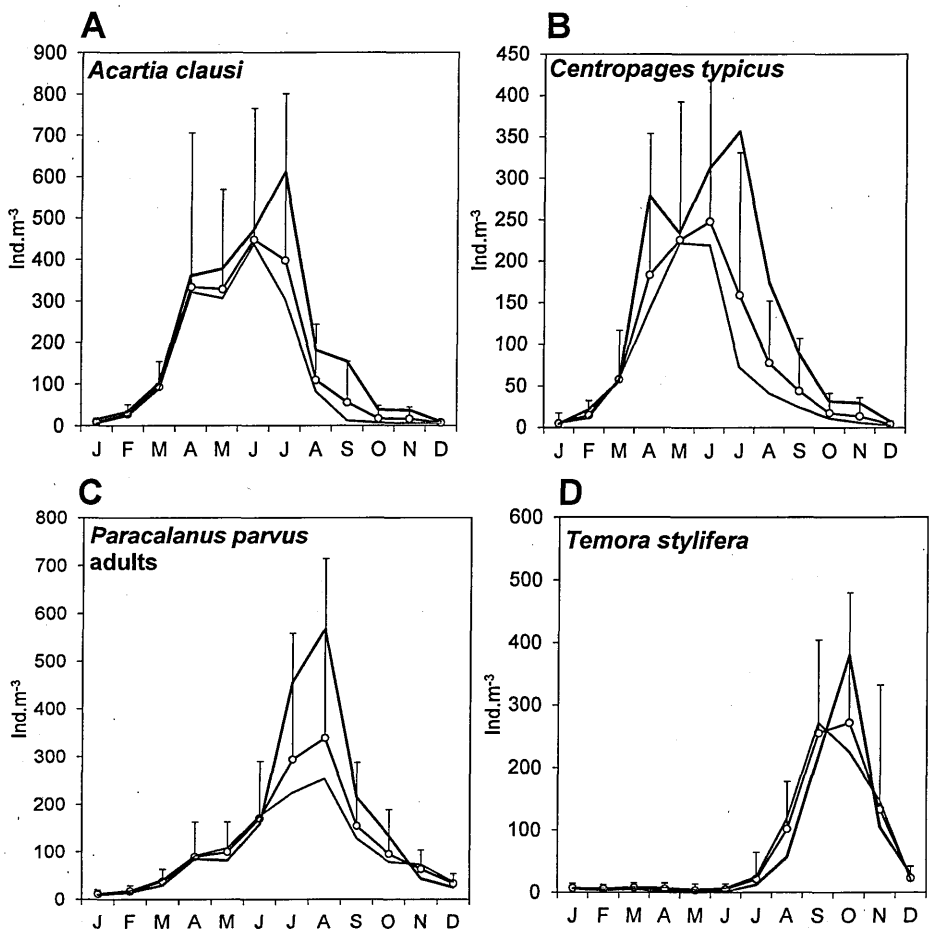

Figure 3.11 Average seasonal cycles of four abundant calanoids: (A) *Acartia clausi,*, (B) *Centropages typicus.* (C) *Paracalanus parvus* adults, (D) *Temora stylifera.* Whole time series (1984-2010) in black) with bars of the standard deviation, first period (1984-1990) in blue, and second period (1995-2010) in red.

The genus *Calocalanus*, which was represented at st. LTER-MC by *C. adriaticus*, *C. contractus, C. neptunus, C. pavo*, *C. pavoninus, C. plumatus, C. plumulosus*, and *C. styliremis* showed a significantly positive trend in long-term abundance in the first (p=0.0376), and in the second (p=0.0171) part of the time series (Table 3.2). The seasonal cycle of this genus was characterized by a primary peak in April and a secondary one in November-December (Fig. 3.12).

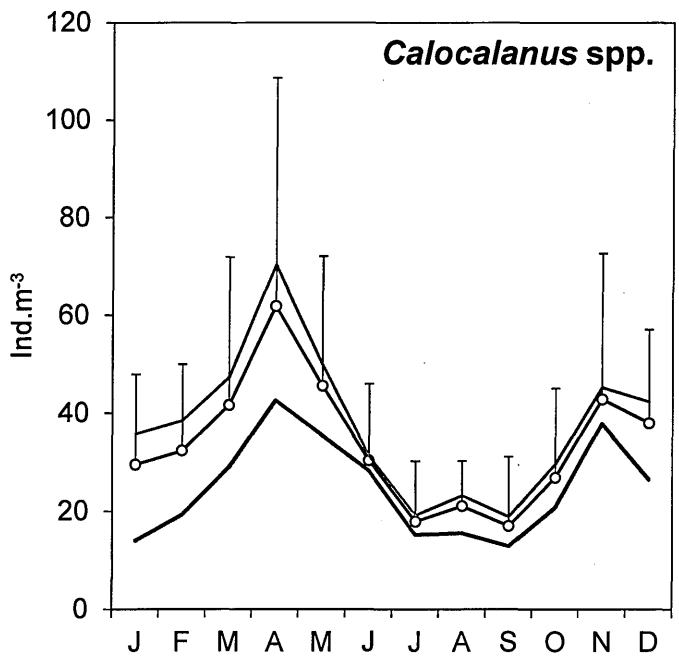

Figure 3.12 Seasonal cycle of abundance of the calanoid genus *Calocalanus* spp. Whole time series (1984-2010) in black) with bars of the standard deviation, first period (1984-1990) in blue, and second period (1995-2010) in red.

The genus *Clausocalanus* occurs with eight species at stn LTER-MC (*C. arcuicornis*, *C. furcatus*, *C. jobei*, *C. lividus*, *C. mastigophorus*, *C. parapergens*, *C. paululus*, *C. pergens*) and their adults contributed 35% to the total annual copepod abundance (Table 3.2). Several of the most abundant representatives of this genus showed shifts in their monthly seasonal peak and overall magnitude of abundance (Fig. 3.13). The abundant category that grouped together the juveniles of *Clausocalanus* spp. and juveniles of *Paracalanus parvus* anticipated the spring peak from April to March and declined in abundance in summer (Fig. 3.13A). *C. furcatus* females changed the overall pattern of seasonal abundance from a bimodal pattern with separated peaks in August and November to a single peak in September (Fig. 3.13B). *C. pergens* displayed a similar change in seasonal abundance, which shifted from being bimodal with peaks in May and July to a single reduced peak in May (Fig. 3.13C). *C. arcuicornis* showed a shift in the timing of seasonal peak abundance nearly a month later from April to May while the secondary peak in August declined in the second part of the time series and the primary peak in May increased (Fig. 3.13D). This species had a significant decreasing trend in abundance in the second part of the time series (Table 3.2). The females of *C. paululus* showed a seasonal cycle that changed from a distinct peak of abundance in April, to a smoother cycle of annual abundance that was highest between January and March (Fig. 3.13E). This species had a significant decreasing trend in abundance in the first part of the time series, similarly to the other small congeneric *C. pergens* (Table 3.2). *Clausocalanus* spp. males shifted the maintained two separated peaks in spring and summer in both parts of the time series, but with a shift from August to September in the summer peak (Fig. 13F). They presented a significant ($p=0.0125$) negative trend in abundance in part I, and a significant positive ($p=0.0077$) trend in mean monthly abundance in part II (Table 3.2).

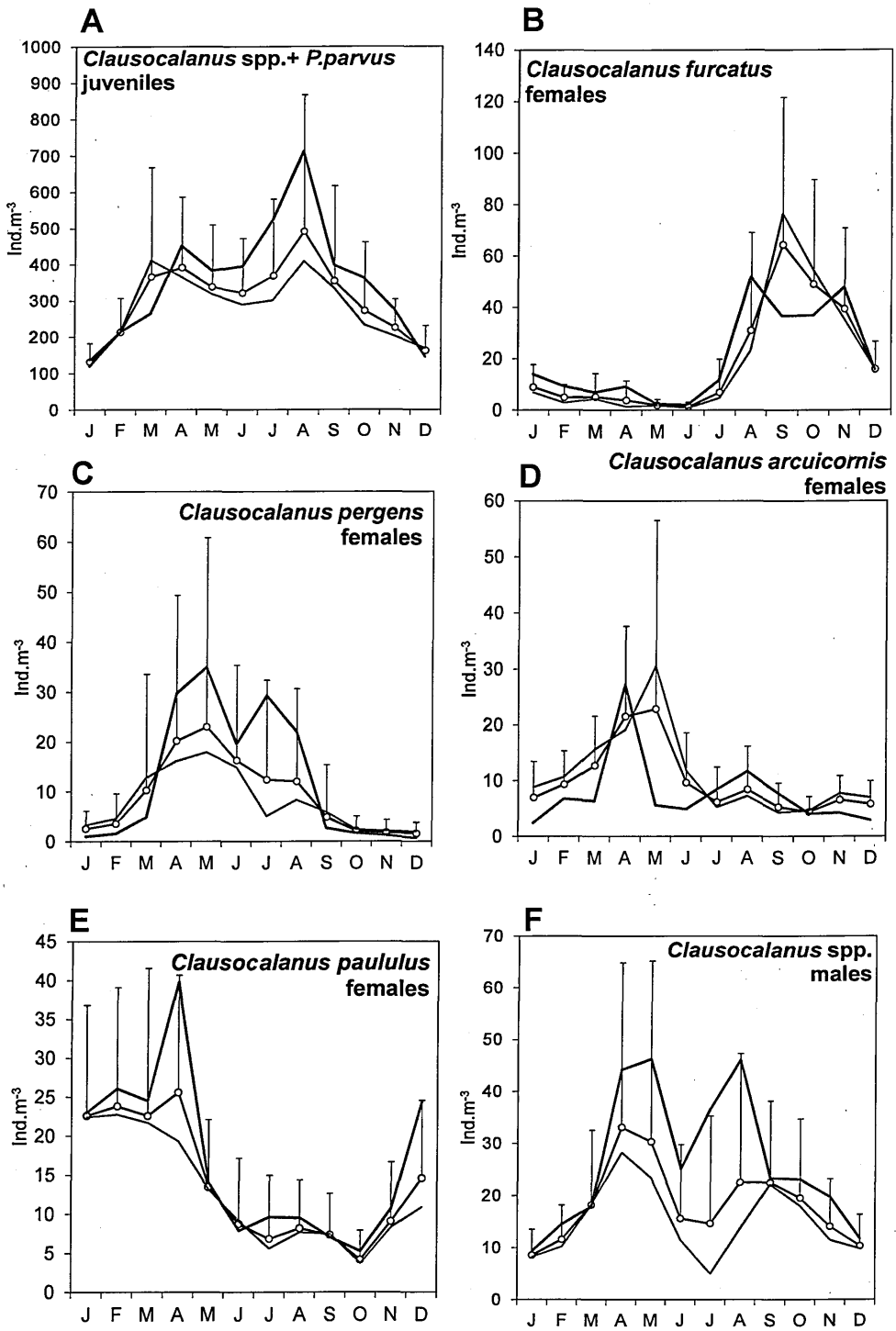

Figure 3.13 Abundant categories within the genus *Clausocalanus* spp.; (A) juveniles of *Clausocalanus* spp. + *P. parvus* females of (B) *C. furcatus,* (C) *C. pergens,* (D) *C. arcuicornis,* (E) *C.paululus* and (F) *Clausocalanus* spp. males. Whole time series (1984-2010) in black) with bars of the standard deviation, first period (1984-1990) in blue, and second period (1995-2010) in red.

Among the abundant and common cyclopoid copepods, several categories within the genus *Oithona* showed change in the seasonal cycle and magnitude of peak abundance (Fig. 3.14). In total, the genus contributed 9.4% to total copepod abundance. *Oithona* spp. males + juveniles showed a distinct bimodal cycle with peaks in April and October (Fig. 3.14A). The two peaks maintained the same timing but in the second part of the time series, the spring peak was lower while the magnitude of the secondary peak in autumn was higher than the first part of the time series. *O. similis* females showed a decline in peak abundance during April (Fig. 3.14B) and a significant ($p=0.0289$), negative trend in abundance in the first part of the time series (Table 3.2). *O. nana* females shifted the month of peak abundance from May to June, and only slightly increased in abundance (Fig. 3.14C), but a significant long term increase in abundance during the first part of the time series (Table 3.2). *O. plumifera* females showed a consistent peak in October with an increase in the second part of the time series (Fig. 3.14D). *O. longispina* females showed a decline in abundance in the second part but it maintained the peak in August-September (Fig. 3.14E).

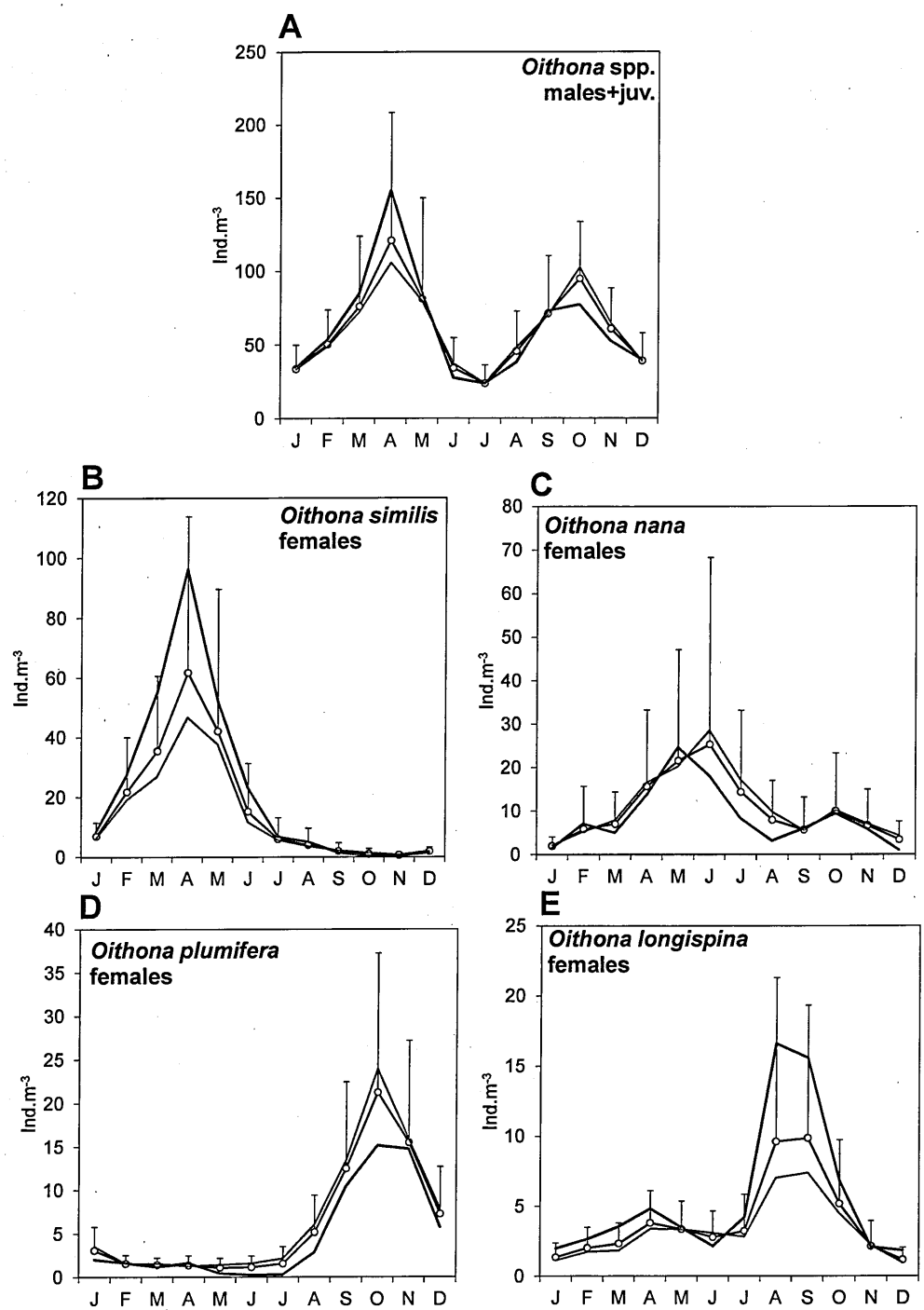

Figure 3.14 Abundant categories within the genus *Oithona*; (A) *Oithona* spp. males + juveniles,, females of (B) *O. similis,*(C) *O. nana,*(D) *O. plumifera,*, and (E) *O. longispina.* Whole time series (1984-2010) in black) with bars of the standard deviation, first period (1984-1990) in blue, and second period (1995-2010) in red.

Among the other zooplanktonic groups, it is worth mentioning cladocerans, which were dominated by *Penilia avirostris*, followed by the group *Evadne spinifera* + *Pseudoevadne tergestina* and the group *Podon intermedius* + *Pleopis polyphemoides* (Fig. 3.15). *P. avirostris* maintained a steep peak in August in both parts of the time series (Fig. 3.15A). *Evadne spinifera* + *Pseudoevadne tergestina* anticipated the month of peak abundance from August to July (Fig. 3.15B). *Podon* + *Pleopis* had a dramatic decline in the abundance from the first to the second part of the time series; in 1995-2010, they maintained the main peak in June while the secondary peak recorded in September in 1984-1990 disappeared (Fig. 3.15C).

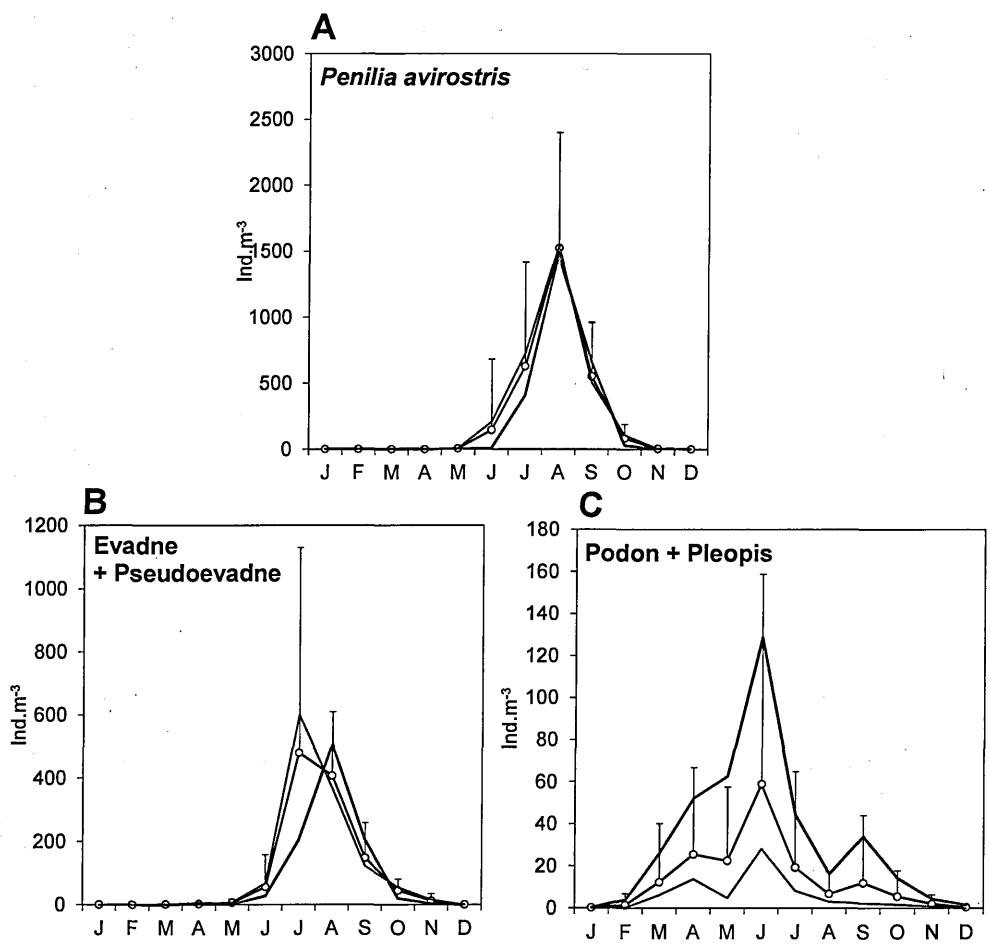

Figure 3.15 Seasonal cycle of abundance for 3 selected categories of cladocerans; (A) *Penilia avirostris,*(B) *Evadne* + *Pseudoevadne,*, and (C) *Podon* + *Pleopis.* Whole time series (1984-2010) in black) with bars of the standard deviation, first period (1984-1990) in blue, and second period (1995-2010) in red.

3.3 Significant long-term changes in phenological events

The number of plankton taxa with significant long-term changes in phenological events (month of seasonal peak, phenophases, duration, and timing of central tendency) varied depending on the type of event and the suitability of the data for testing. Overall, only 25 out of 104 phytoplankton taxa could be tested for change in phenophases as compared to 50 out of 88 zooplankton (Table 3.3). Significantly more phytoplankton and zooplankton taxa could be tested for trends in duration and timing. The timing of central tendency proved to be the most reliable phenological index that could be applied to phytoplankton taxa.

27% of phytoplankton taxa showed a change in the month of seasonal peak, as compared to 30% of zooplankton, 32% of phytoplankton and 36% of zooplankton taxa showed a change in phenophases, 11% of phytoplankton had trends in duration while zooplankton had two-fold number of trends (22%), and both communities showed a similar relationship of change in timing; 21% of the phytoplankton taxa, and 20% of zooplankton had trends in the timing of central tendency. The overall changes in different phenological events are herein described for priority taxa that are numerically abundant or that represented an important functional group within the plankton communities.

Table 3.3 Percentage of phytoplankton and zooplankton categories with significant trends in phenological events, and the corresponding number (n) of categories tested.

phenological event	phytoplankton n=104	zooplankton n=88
seasonal peak	27% n=104	30% n=88
onset (start 25%)	28% n=25	22% n=50
peak (middle 50%)	12% n=25	22% n=50
end (end 75%)	4% n=25	16% n=50
duration	11% n=81	22% n=74
timing	21% n=78	20% n=88

Table 3.4 Plankton categories (A) phytoplankton and (B) zooplankton with significant long term changes in phenophases. Within each group, taxa are listed alphabetically. The type of change in phenology is indicated as earlier or later. The specific phenophase considers start, middle and end (25, 50 and 75% cumulative percentile abundance) respectively. Colored circles indicate a significant trend in more than 1 phenophase for phyplankton (green) and zooplankton (red).

	phytoplankton	change	phenophase	time series†	p-value
diatoms	O *Chaetoceros socialis*	later	start	II	0.0196*
	"	later	middle	II	0.0033**
	Chaetoceros tenuissiumus	later	start	II	0.0417*
	Proboscia alata	earlier	start	I	0.0483*
	O *Minidiscus comicus*	earlier	start	II	0.0011**
	"	earlier	middle	II	0.0001***
	"	earlier	end	II	0.0005**
	Skeletonema menzelii	later	start	II	0.0226*
dinoflagellate	*Dinobryon faculiferum*	later	start	II	0.0013**
dinoflagellate	*Heterocapsa niei*	earlier	start	II	<0.001***
other flagellate	*Pachysphaera* spp.	later	middle	II	0.0380*
	zooplankton				
bulk	Bivalve larvae	later	start	II	0.0406*
	Chaetognaths	later	start	II	0.0372*
	O salps	later	middle	I	0.0386*
	"	later	end	I	0.0026**
	"	later	start	II	0.0175*
cladocerans	O *Podon + Pleopis*	earlier	start	I	0.0116*
	"	earlier	middle	I	0.0465*
	"	earlier	end	I	0.0143*
copepods	O *Acartia clausi*	earlier	start	II	0.0118*
	"	earlier	middle	II	0.0063**
	"	earlier	end	II	0.0127*
	Centropages kroyeri	earlier	middle	I	0.0435*
	Centropages violaceus	later	middle	II	0.0247*
	Clausocalanus arcuicornis (females)	earlier	middle	II	0.0430*
	Clausocalanus furcatus (females)	later	start	II	0.0378*
	Clausocalanus lividus (females)	later	start	I	0.0025**
	O Eucalanidae	later	start	I	0.0329*
	"	later	middle	I	0.0080**
	"	later	end	I	0.0114*
	O *Isias clavipes*	earlier	middle	I	0.0292*
	"	earlier	end	I	0.0534*
	"	earlier	start	II	0.0200*
	Microsetella spp.	earlier	middle	I	0.0263*
	Oithona longispina	earlier	start	I	0.0255
	O *Oithona plumifera*	later	middle	II	0.0273**
	"	later	end	II	0.0309*
	Oithona setigera females	earlier	start	I	0.0255*
	O *Oithona similis* (females)	earlier	start	I	0.0080**
	"	earlier	middle	I	0.0002**
	"	earlier	end	I	0.0002**
	Paracalanus denudatus	earlier	end	I	0.0257*

† Time series Part I (1984-1990) and Part II (1995-2010)

3.3.1. Phenophases

Only 24% of phytoplankton categories could be statistically tested for trends in their phenophases due to seasonal variability, missing years of data or otherwise a bimodal cycle of abundance that prevents using the cumulative percentile method to identify phenology of some taxa. In total, only 25 categories had sufficient years of data to test phenophases. Only 8 of the 25 categories showed significant trends (Table 3.4).

Significant changes in the phenophases were recorded in two abundant spring diatoms (*Chaetoceros socialis* and *Chaetoceros tenuissimus*) which both displayed a late onset of phenology (start phenophase) in the second part of the time series (Fig. 3.16). In 1995, the start phenophase occurred at day 52 and 63 for *C. socialis* and *C. tenuissimus* respectively. The start phenophase was significantly late for both species from 1995 to 2010 however this trend could be attributed to the earlier than average start day in 1995. On average, the mean start phenophase was a difference of 10 days between a mean of day 131 for *C. socialis* and day 141 for *C. tenuissimus*. In general, the start phenophase of these two diatom species was significantly later and the general pattern of these phenological events showed a trend towards a shorter phenological season.

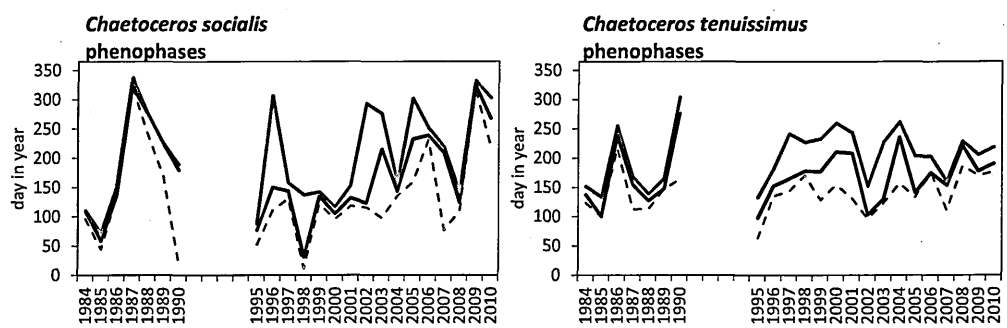

Figure 3.16. Long-term changes in phenophases of two spring diatom species *Chaetoceros socialis* and *C. tenuissimus*. Start (dashed), middle (black), and end (red) day in the year calculated as 25, 50, 75% of cumulative abundance, respectively.

In contrast, two summer diatom species, i.e., *Minidiscus comicus* and *Skeletonema menzelii,* had earlier phenophases in the second part of the time series (Fig. 3.17). The start phenophase for *M. comicus* varied from day 294 in 1995, to day 51 in 2010. *M. comicus* also had an unusual pattern of phenology which abruptly changed in year 2003. The overall change was significantly earlier for the start, middle and end phenophases. *S. menzelii* had a significantly earlier trend (p=0.0226) only in the start phenophase (Fig. 3.17). Overall, *S. menzelii* had a variable pattern of phenophases which were earlier and at the end of the time series, the phenology of this species appeared very short.

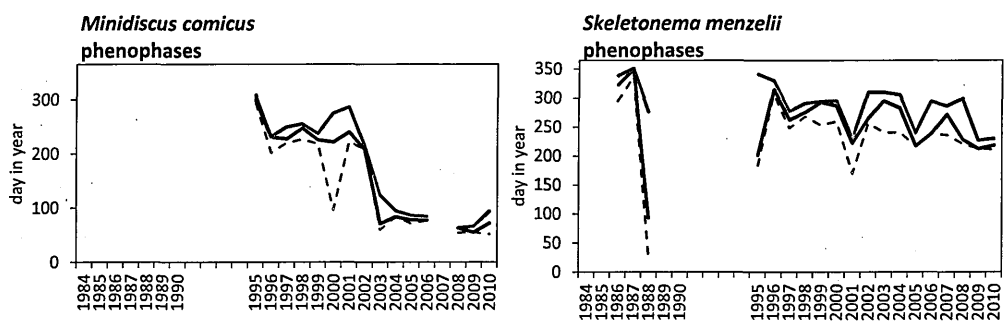

Figure 3.17. Phenophases of two summer diatom taxa; *Minidiscus comicus* and *Skeletonema menzelii* which had significantly earlier trends in phenophases from 1995 to 2010. The phenophases are not presented for *M.comicus* in the first part of the time series. Linear trend analysis was applied to phenophases from 1995 to 2010.

Within zooplankton, 17 taxa had significant long-term trends in phenophases (Table 3.4), but only 7 of them contributed more than 0.5% to total community abundance, i.e., the calanoid copepods *A. clausi* (14.5%), *C. furcatus* (1.8%), *C. arcuicornis* (1.0%), the cyclopoid copepods *O. similis* (1.5%), *O. plumifera* (0.6%), the cladocerans *Podon+ Pleopis* (4.6%), and the group of chaetognaths (0.5%).

Acartia clausi showed significant trends in all phenophases in the second part of the time series, with an anticipation of the start phenophase from May (day 137) to April (day 106), and the end phenophase from July (day 199) to June (day 155) (Fig. 3.18A). *C. arcuicornis*, with a spring onset, had a significantly (p=0.0430) anticipated middle phase of

the season from day 136 in the first part to day 125 in the second part of the time series (Fig. 3.18B). Conversely, the congeneric autumnal *C. furcatus* had a significantly ($p=0.0378$) later start of the season from day 67 in 1995 to day 282 in 2010 (Fig. 3.18C). The trend observed in the second phase of the time series was clearly shaped by the unusual anticipated onset in 1995. The spring copepod *O. similis* had a significant anticipation of all phenophases in the first part of the time series (Fig. 3.18D), whereas the autumnal *O. plumifera* had significantly later middle and end phases in the second part (Fig. 3.18E). Among other zooplanktonic groups, the cladoceran category comprised of *Podon* and *Pleopis* had a significant anticipation in all phenophases from 1984 to 1990 and afterwards showed high interannual variability in phenophases without significant trends (Fig. 3.18F). Chaetognaths had a significantly earlier trend only in the start phenophase, which shifted from day 118 in 1995 to day 183 in 2010 (Fig. 3.18G).

Other less abundant zooplankton taxa showed significant changes in different phases of their phenology (Table 3.4), such as the copepod species *Centropages kroyeri and C. violaceus, Clausocalanus lividus, O. setigera* or the family Eucalanidae whose phenophases were all significantly later in the first part of the time series, but they were too variable after 1995 to extract a regular phenology. Salps had significantly later trends in all phenophases, while chaetognaths and bivalve larve only in the start phase (Table 3.4).

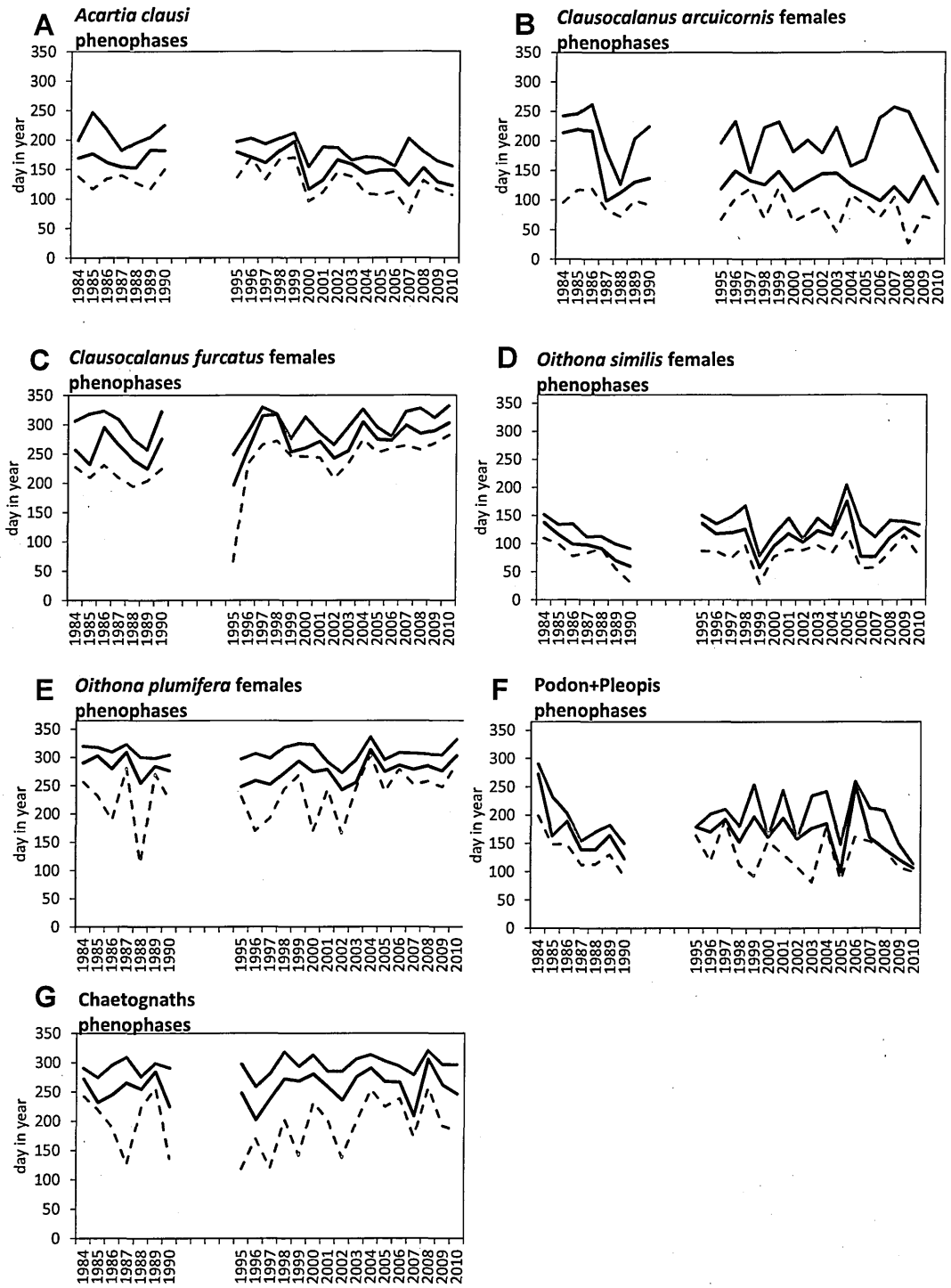

Figure 3.18 Zooplankton taxa with significant trends in phenophases; (A) *Acartia clausi*, (B) *Clausocalanus arcuicornis* females, (C) *Clausocalanus furcatus* females, (D) *Oithona similis* females, (E) *Oithona plumifera* females, (F) *Podon+Pleopis*, and (G) chaetognaths.

3.3.2. Duration

The *duration*, or length of phenological season (expressed as the difference in number of days between the end and start phenophases) did not show significant long-term changes for the overall phyto- and zooplankton communities. However, a few taxa and important groups had significantly shorter or longer duration (Table 3.5).

Table 3.5 Plankton categories (A) phytoplankton and (B) zooplankton with significant change in the length of seasonal duration calculated as the difference (number of days) from the end to the start of the season within each of the two parts of the time series.

A phytoplankton	change	difference (days)	time series†	p-value
Total dinoflagellates	shorter	-80	I	0.0285*
Chaetoceros affinis	longer	106	I	0.0028**
Chaetoceros anastomosans	longer	105	I	0.0203*
Chaetoceros simplex	longer	42	II	0.0101**
Dactyliosolen phuketensis	shorter	-95	II	0.0128*
Thalassiosira mediterranea	longer	138	I	0.0013**
Thalassiosira spp.	longer	169	I	0.0263*
pennate diatoms >10mm	longer	129	II	0.0434*
undetermined coccolithophores	shorter	-49	I	0.0366*
B zooplankton				
Bivalve larvae	shorter	-11	II	0.0543
Chaetognaths	shorter	-66	II	0.0406*
Gastropods (larvae + pteropods)	longer	197	II	0.0050**
Hydromedusae	shorter	-168	II	0.0279*
Salps	longer	-106	I	0.0138*
Acartia negligens	longer	56	II	0.0143*
Calanidae juveniles	shorter	-167	II	0.0386*
Calanoid juveniles	longer	4	I	0.0015**
Calocalanus spp.	longer	108	II	0.0036**
Centropages ponticus	longer	29	I	0.0483*
Clytemnestra rostrata	shorter	-115	I	0.0472*
Corycaeus spp.	shorter	-105	I	0.0278*
Oithona nana	longer	66	II	0.0027**
Oithona plumifera	shorter	-24	II	0.0215*
Scaphocalanus spp.	shorter	-138	I	0.0381*
Temora stylifera	shorter	-7	II	0.0279*

† Time series considers part I: 1984-1990 and part II: 1995-2010

Within phytoplankton, total dinoflagellates had a significant (p=0.0285) negative trend in duration which was 80 days shorter from a length of 139 days in 1984 to 59 days in 1990 (Fig. 3.19A). After 1995, the duration was variable but ranged from 77 to 69 days in length between 1995 and 2010. Undetermined coccolithophores had a significantly (p=0.0366) shorter duration from 77 days to 28 days in the first period (Fig. 3.19B). The duration in the second period increased and was an average length of 56 days. Among diatoms, *Chaetoceros affinis, C. anastomosans, Thalassiosira* spp. and *T. mediterranea* had significantly longer trends in their phenological season. In particular, the two species of *Chaetoceros* had sporadic patterns of phenology. Thus, the pattern of duration that emerged was a result of abundance records that measured the start phenophase but were missing the middle of end phases to calculate duration (Fig. 3.19C, D). The variable pattern observed in *Thalassiosira* spp. duration was shaped by the contribution of unidentified species that comprise this category (Fig. 3.19E). *T. mediterranea* showed a pattern of decreased duration from 1995 to 1997 followed by 4 years where the duration stabilized and afterwards remained variable and sporadic (Fig. 3.19F). In the second part of the time series, the patterns of duration of these four taxa became more variable and did not show any significant trends in long-term duration over the 16 year period.

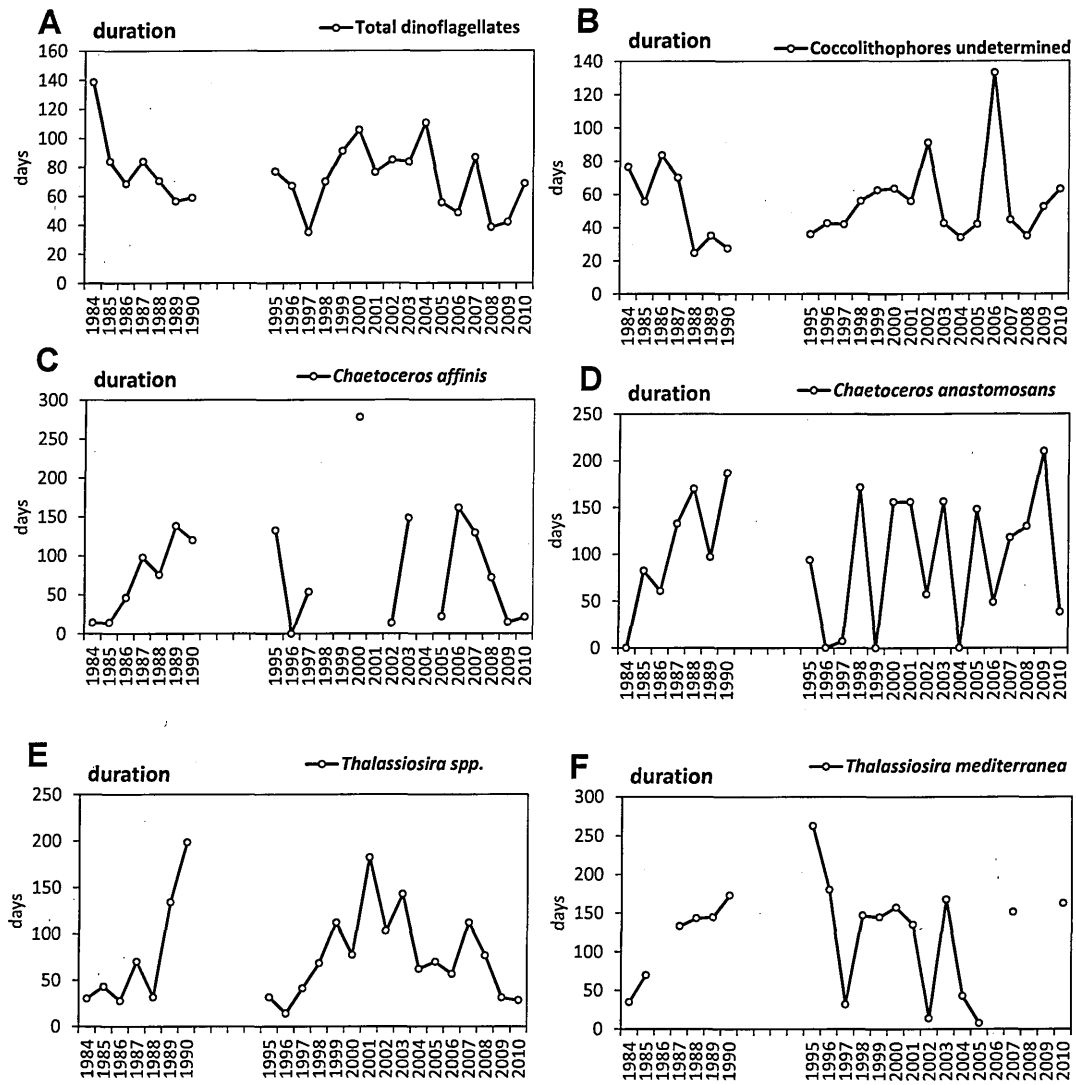

Figure 3.19. Phytoplankton taxa with significant trends in duration in the first part of the time series (1984-1990); (A) total dinoflagellates, (B) coccolithophores undetermined, (C) *Chaetoceros affinis*, (D) *Chaetoceros anastomosans*, (E) *Thalassiosira* spp., and (F) *Thalassiosira mediterranea.*

In the second part of the time series, the diatom *Dactyliosolen phuketensis* had a significantly (p=0.0128) shorter duration that changed from over 100 days from 1995 to 1999 to 31 days in 2000, and 35 days in 2010, with the exception of 2006 in which the duration lasted 154 days (Fig. 3.20A). Overall, the change in duration was 95 days shorter. Conversely, the diatom *Chaetoceros simplex* showed a gradual lengthening of seasonal duration with a significant trend (p=0.0101) starting in 1997 (Fig. 3.20B). Pennate diatoms >10μm had a significantly longer duration from 1995 to 2010 (Fig. 3.20C). The duration of this latter category, which is composed of an unknown number of different species grouped by size, was highly variable and ranged from a length of 63 days in 1995 to 192 in 2010.

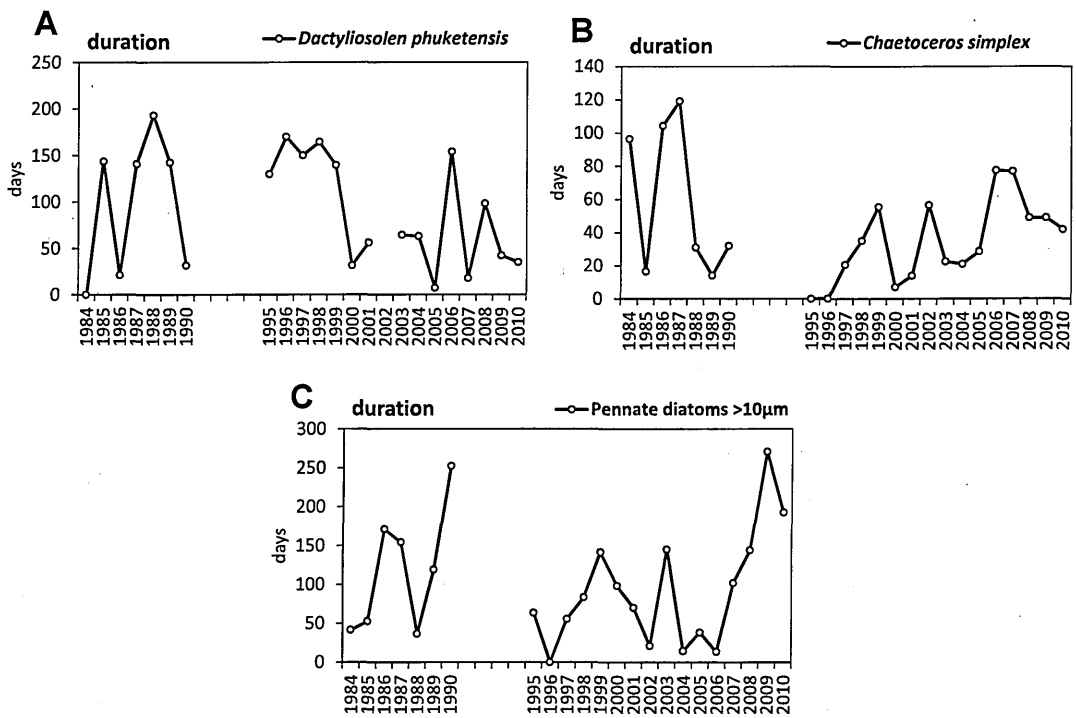

Figure 3.20 Duration of phytoplankton categories with significant trends in the second part of the time series; (A) *Dactyliosolen phuketensis*, (B) *Chaetoceros socialis*, and (C) pennate diatoms > 10 μm.

Several zooplankton taxa had significant trends in the duration of their annual cycle, but only a few taxa among the most abundant ones (Table 3.4). From 1984 to 1990, the abundant category of calanoid juveniles (juveniles of *Clausocalanus* spp. + *P. parvus*) and salps had a significantly longer duration (Fig. 3.21A, B). From 1995 to 2010, the seasonal duration was significantly shorter for *T. stylifera* (-7 days), *O. plumifera* (-24 days), bivalve larvae (-11 days) and chaetognaths (-66 days) (Fig 3.21C-F). Conversely, the duration of the season became significantly longer for the copepods *Calocalanus* spp. (+108 days) and *O. nana* (+66 days), and for the gastropods (+ 197 days).

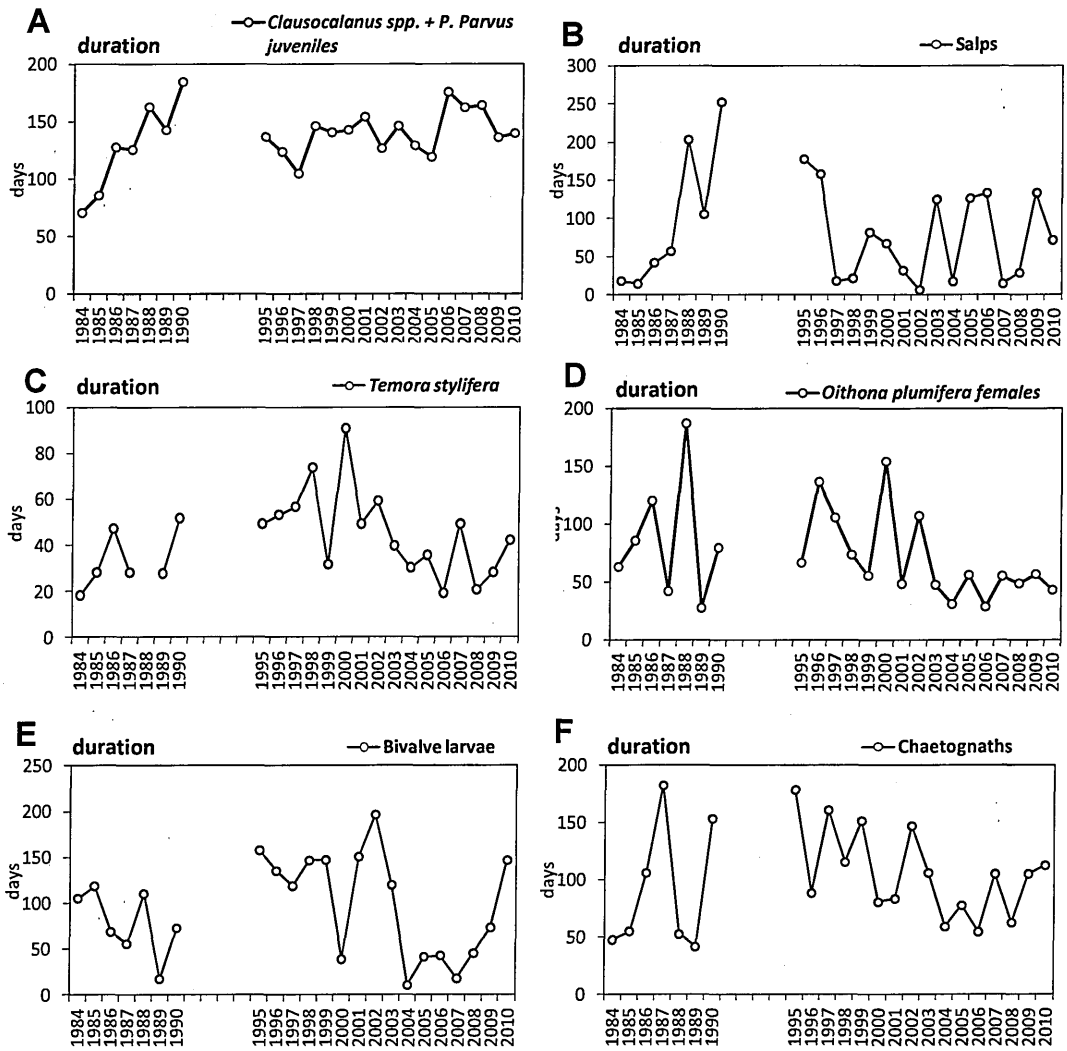

Figure 3.21. Duration of abundant zooplankton categories with significant trends (A) *Clausocalanus* spp + *P.parvus* juveniles, (B) salps, (C) *Temora stylifera* (D), *Oithona plumifera* females, (E) bivalve larvae, and (F) chaetognaths.

Overall, significant changes in duration were observed more numerous in zooplanktonic taxa that peaked in late summer-autumn (*T. stylifera, O. plumifera*, chaetognaths) than in spring (bivalve larvae). There were no categories that had a trend in both parts of the time series. Chaetognaths, bivalve larvae, and gastropods were the only categories that had a concurrent pattern of later end phenophases and shorter duration.

3.3.3. Timing of central tendency

The most numerous changes in phenological events occurred in the timing of central tendency (Table 3.6). Timing of groups with multiple taxa or species with bimodal seasonality (as identified in previous portion of this chapter) was determined in two different parts of the year.

Among bulk phytoplankton categories, significant trends occurred primarily in the second part of the time series and in the second half of the year (Table 3.6A). Total diatoms had significantly earlier (p=0.0222) timing from 1995 to 2010; the trend reflected a gradual anticipation from the early part of August in 1995 towards the end of July in 2010 (Fig. 3.22A). Total dinoflagellates had a significantly earlier (p=0.0171) trend in the first period reflecting a change in timing from mid-September to August (Fig. 3.22B). Total coccolithophores showed a significantly later trend in timing (p=0.0370) in the first period of the time series (Fig. 3.22C).

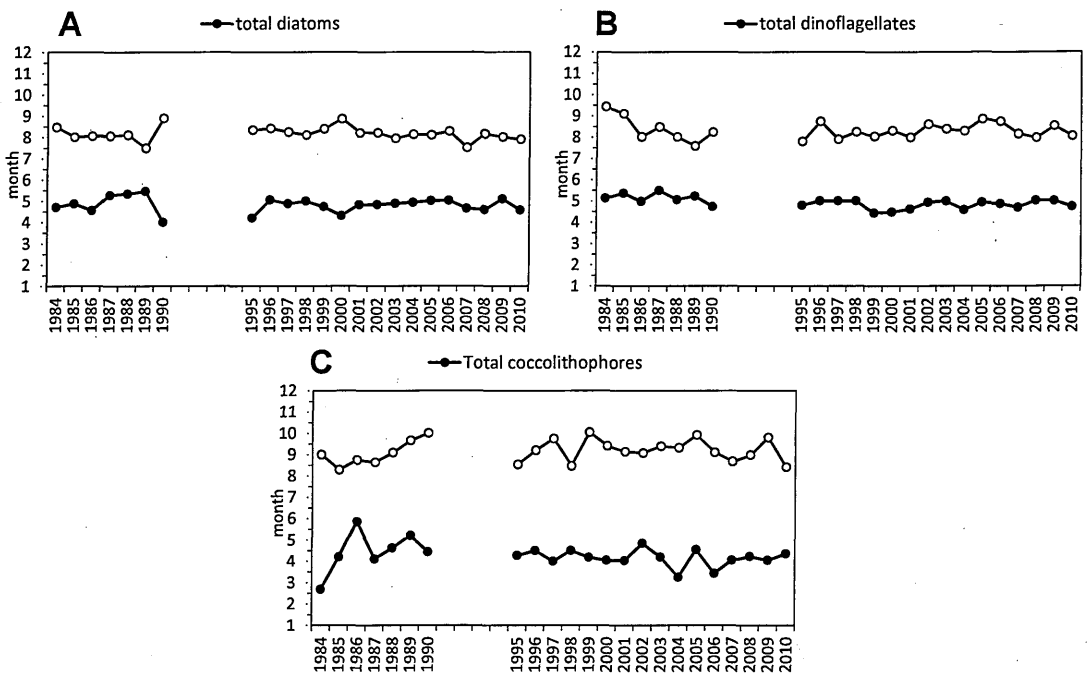

Figure 3.22. Bulk plankton categories (A) total diatoms, (B) total dinoflagellates, and (C) total coccolithophores with significant long-term changes in the seasonal timing of central tendency. The timing of phytoplankton categories is shown in each of the two parts of the annual cycle; filled black circles from January to June (1:6), and open circles from July to December (7:12).

Table 3.6 Plankton categories; (A) phytoplankton and (B) zooplankton with significant long term changes in timing of central tendency. The type of change in timing was determined by the slope of linear trend as positive or negative (earlier). Significance values are based on $p \leq 0.05$.

A	phytoplankton	change	timing	time series†	p-value
bulk	total diatoms	earlier	T [7:12]	II	0.0222*
	total dinoflagellates	earlier	T [7:12]	I	0.0369*
	total coccolithophores	later	T [7:12]	I	0.0328*
diatoms	*Chaetoceros socialis*	later	T [1:12]	II	0.0132*
	Chaetoceros tenuissiumus	earlier	T [7:12]	II	0.0014**
	Minidiscus comicus	earlier	T [1:12]	II	0.0002***
	Pseudo-nitzschia multistriata	earlier	T [1:12]	II	0.0427*
	Skeletonema menzelii	earlier	T [1:12]	II	0.0030**
	Thalassiosira mediterranea	earlier	T [1:12]	II	0.0453*
	Thalassionema nitzschioides	later	T [1:12]	I	0.0381*
	Thalassiosira spp.	earlier	T [1:6]	I	0.0201*
	Undetermined phytoflagellates >10µm	later	T [1:6]	II	0.0115*
dinoflagellates	*Heterocapsa niei*	earlier	T [1:12]	II	0.0005***
	Naked dinoflagellates>15µm	earlier	T [7:12]	I	0.0055**
other flagellates	*Diplostauron cf.elegans*	earlier	T [7:12]	II	0.0161*
coccolithophores	*Emiliana huxleyi*	later	T [7:12]	I	0.0364*

B	zooplankton	change	timing	time series†	p-value
bulk	Appendicularians	earlier	T [1:6]	I	0.0297*
	Isopod Epicaridea larvae	earlier	T [1:12]	I	0.0026**
	Ostracods	earlier	T [1:6]	II	0.0034**
	salps	later	T [1:12]	I	0.0108*
	Total copepods	earlier	T [1:6]	I	0.0278*
	Total zooplankton	earlier	T [1:6]	I	0.0382*
	"	earlier	T [1:6]	II	0.0014**
cladocerans	Podon + Pleopis	earlier	T [1:12]	I	0.0337*
copepods	Calanidae juveniles	earlier	T [1:6]	I	0.0405*
	Clausocalanus mastigophorus females	earlier	T [7:12]	I	0.0312*
	Clausocalanus paululus	earlier	T [1:12]	II	0.0168*
	Clausocalanus spp. (males)	earlier	T [1:6]	I	0.0008
	Farranula rostrata	earlier	T [1:6]	II	0.0056**
	Isias clavipes	earlier	T [1:12]	I	0.0304*
	Nannocalanus minor	earlier	T [7:12]	II	0.0099*
	Oithona similis (females)	earlier	T [1:12]	I	0.0003**
	Oithona setigera females	earlier	T [1:12]	II	0.0360*
	Oithona spp. males+juv.	earlier	T [1:6]	I	0.0027**
	Oithona spp. males+juv.	earlier	T [1:6]	II	0.0013**
	Scolecithricella spp.	earlier	T [1:6]	I	0.0106*

At species-level, several numerically dominant diatoms had significantly later timing (Fig. 3.23). *C. socialis* changed the timing from mid-March in 1995 towards the end of August in 2010 (Fig. 3.23A) and *C. tenuissimus* changed from the end of March in 1995 to early May in 2010 (Fig. 3.23B). Two diatoms with peak abundance in summer had significantly earlier timing from 1995 to 2010. *Pseudo-nitzschia multistriata* was not recorded at LTER-MC until the second part of the time series. This species occurred in February in 1995, but in years afterwards its timing ranged between early October in 1996, to mid-July in 2010 (Fig. 3.23C). Although the earlier trend was shaped by a significantly early occurrence in 1995, the trend was still significantly earlier from 1996 to 2010. *Skeletonema menzelli* changed in timing from November in 1995 to August in 2010 (Fig. 3.23D). *Thalassiosira mediterranea* had a variable timing of central tendency, but the overall trend was earlier (Fig. 3.23E).

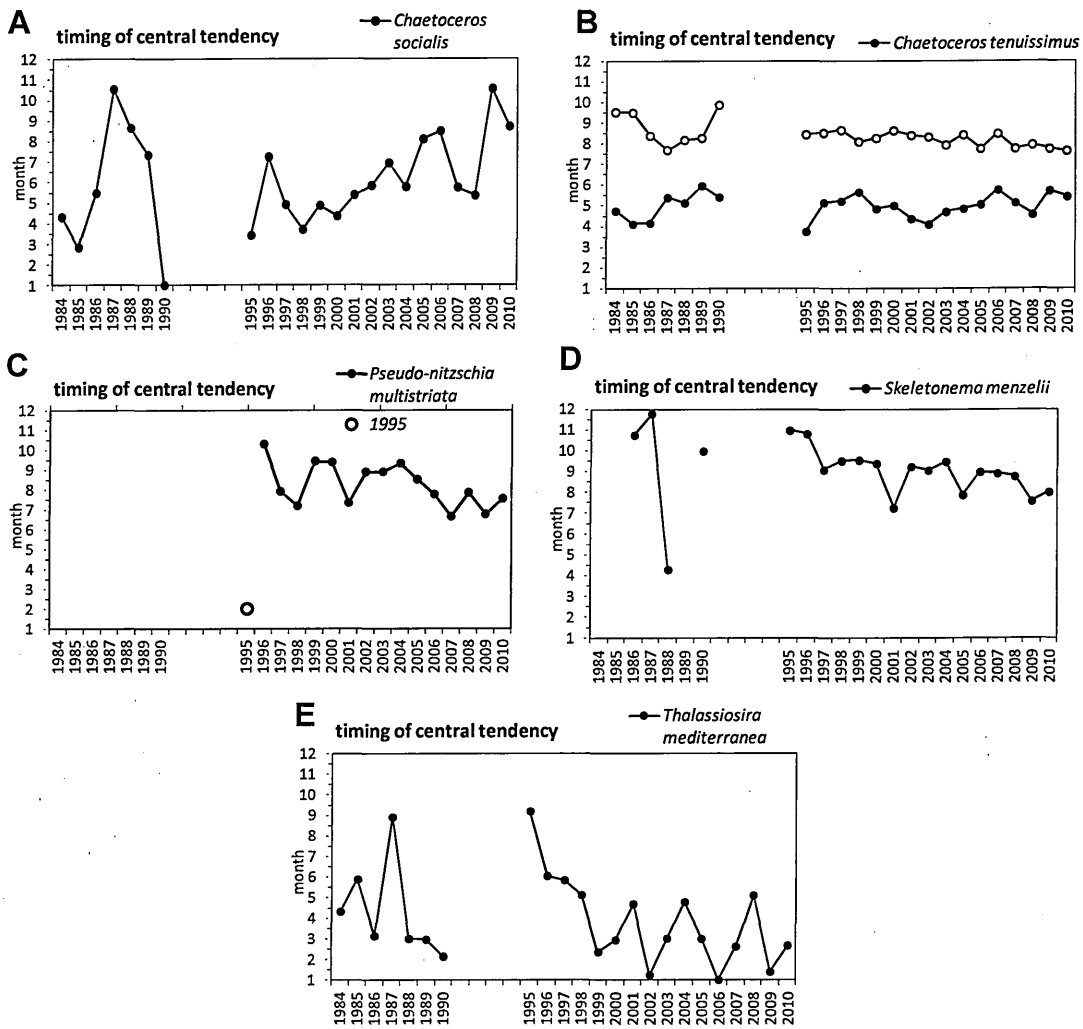

Figure 3.23. Selected phytoplankton taxa with significant long-term changes in the seasonal timing of central tendency; (A) *Chaetoceros socialis,* (B) *Chaetoceros tenuissimus,* (C) *Pseudo-nitzschia multistriata,* (D) *Skeletonema menzelii,* and (E) *Thalassiosira mediterranea.* Open circle represents a year excluded from trend analysis.

Among diatoms, four categories showed notable changes in phenology though in some cases without significant trends (Fig. 3.24). *Minidiscus comicus* occurred between July and October from 1995 to 2002, and then between late February to early March for years thereafter. This abrupt change in phenology shaped the long-term trend in earlier timing in the second part of the time series. The timing of pennate diatoms >10μm in the first half of the year ranged from early May in 1995 to February in 2010, without any significant trend. The timing occurred between mid May and late April from 1995 to 2001; it was much earlier in 2002 and maintained a gradually later pattern from 2003 to 2010 (Fig. 3.24). The spring dinoflagellate *Dinobryon faculiferum* had a significantly later trend in the start phenophase and had a shorter duration, however neither the timing nor long-term duration changed significantly.

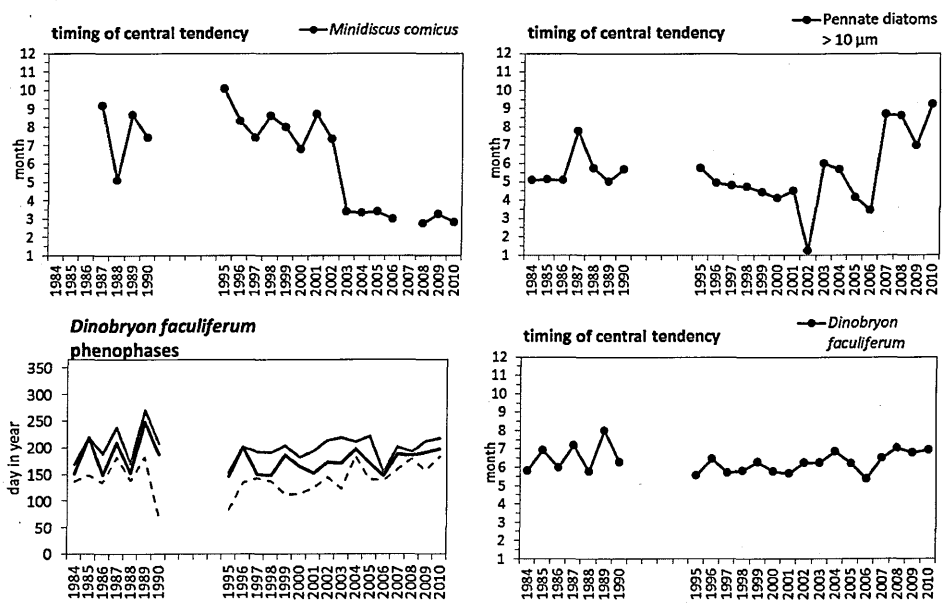

Figure 3.24. Selected phytoplankton categories with abrupt changes in their long-term phenological timing or events.

Total zooplankton timing from January to June was significantly earlier in both parts of the time series (Fig. 3.25A). Among the most abundant zooplankton groups, total copepods had a significantly earlier ($p=0.0262$, $n=7$) trend in the first part of the time series (Fig. 3.25B). Additionally, appendicularians were significantly earlier ($p=0.0398$, $n=7$) in the first part of the time series (Fig. 3.25C). Cirriped larvae showed a trend towards earlier timing in the first part of the time series from March to February, however the timing was not significant (Fig. 3.25D). Other groups such as cladocerans, ostracods, gastropod larvae, and salps also changed their long-term timing (Table 3.6B).

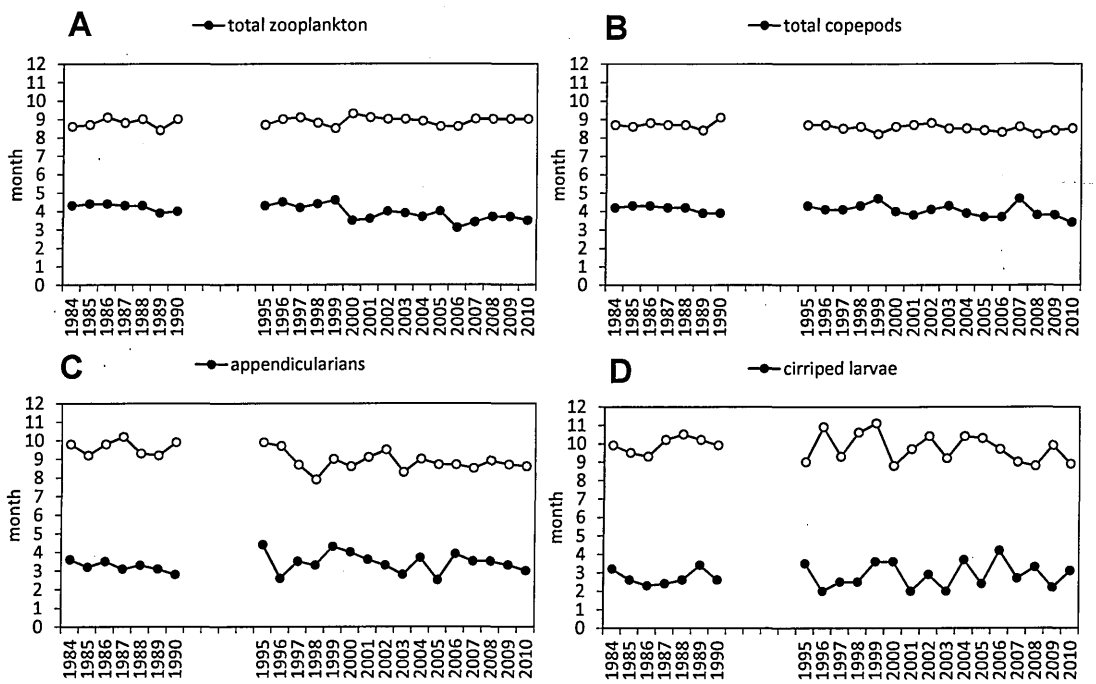

Figure 3.25. Selected zooplankton taxa (A) total zooplankton, (B) total copepods, (C) appendicularians, and (D) cirriped larvae with significant long-term changes in the seasonal timing of central tendency. The timing of phytoplankton categories is shown in each of the two parts of the annual cycle; filled black circles from January to June (1:6), and open circles from July to December (7:12).

Among copepod species, *Oithona* spp. males and juveniles had a consistent earlier timing from April to March in the first and second part of the time series, whereas the timing in the second portion of the year remained stable in September (Fig. 3.26A). *Oithona similis* had an earlier trend in timing from 1984 to 1990, but this trend did not

continue until 2010 (Fig. 3.26B). *Clausocalanus paululus* females had a negative trend (p=0.0475) which began with a pattern of earlier timing after 2000 (Fig. 3.26C). The bimodal species *Farranula rostrata* had a significantly earlier timing from March to February in the first half of the year (Fig. 3.26D). The spring copepod *Isias clavipes* was significantly earlier (p=0.0304, n=16) trend in timing from May to April (Fig. 3.26E).

Overall, numerous spring and summer taxa within the zooplankton community had an earlier timing but only two maintained the same long-term pattern in timing in both parts of the time series, i.e., total zooplankton, *Oithona* spp. and males+juveniles (Table 3.6B).

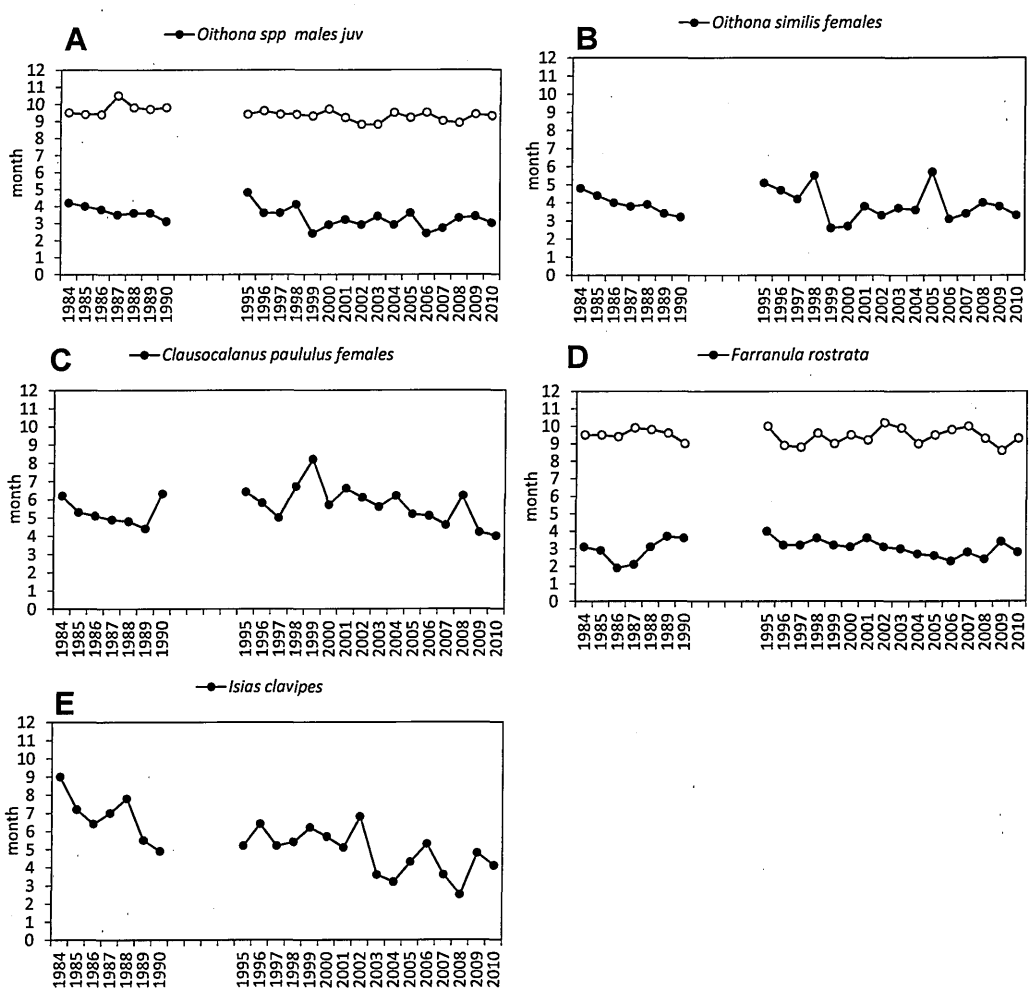

Figure 3.26 Selected abundant copepod taxa (A) *Oithona spp.* males + juveniles, (B) *Oithona similis* females, (C) *Clausocalanus paululus* females, (D), *Farranula rostrata*, and (F) *Isias clavipes* which had significant trends in timing of central tendency. The timing of (B) and (E) is shown in each of the two parts of the annual cycle; filled black circles from January to June (1:6), and open circles from July to December (7:12).

3.4 Long-term patterns of chlorophyll *a* phenology

Phenological events were analyzed for surface and depth integrated chl *a* concentrations (Fig. 3.27). Surface chl *a* onset was delayed with respect to integrated chl *a* with an average onset (start phenophase) of day 122 (surface) as compared to day 93 (integrated) (Fig 3.27A). Integrated phenophases showed an earlier average onset of day 93 and later end phenophase average on day 261 (Fig 3.27B). Both surface and integrated start phenophases were a difference of 10 days earlier between 1984 and 2010.

The duration of surface and integrated chl *a* was variable in the first period (Fig. 3.27C, D) and appeared to lengthen in the second period; however there was no significant trend in surface ($p=0.9601$) or integrated chl *a* ($p=0.4389$). The average duration of integrated chl *a* was 54 days longer than the duration of surface chl *a*.

Chl *a* timing of central tendency did not significantly change in either part of the time series (Fig. 3.27 E, F). Surface chl *a* showed two peak seasons of occurrence in early April in the first half of the year, and mid September in the second half of the year (Fig. 3.27E). An exceptionally early timing of the second peak in mid July occurred in 1989. Integrated chl *a* timing generally occurred between mid March and April, and from September to October (Fig. 3.27F).

In summary, chl *a* phenology did not showed variability, with the exception of the start and middle phenophases of surface chl *a* which showed a trend only due to the inclusion of year 1995. When 1995 was removed, the trend that was apparent in the start and middle phenophases was no longer significant. Surface chl *a* occurred later than integrated chl *a* in all phenophases and peak timing, and the duration of integrated chl *a* seemed to be extending.

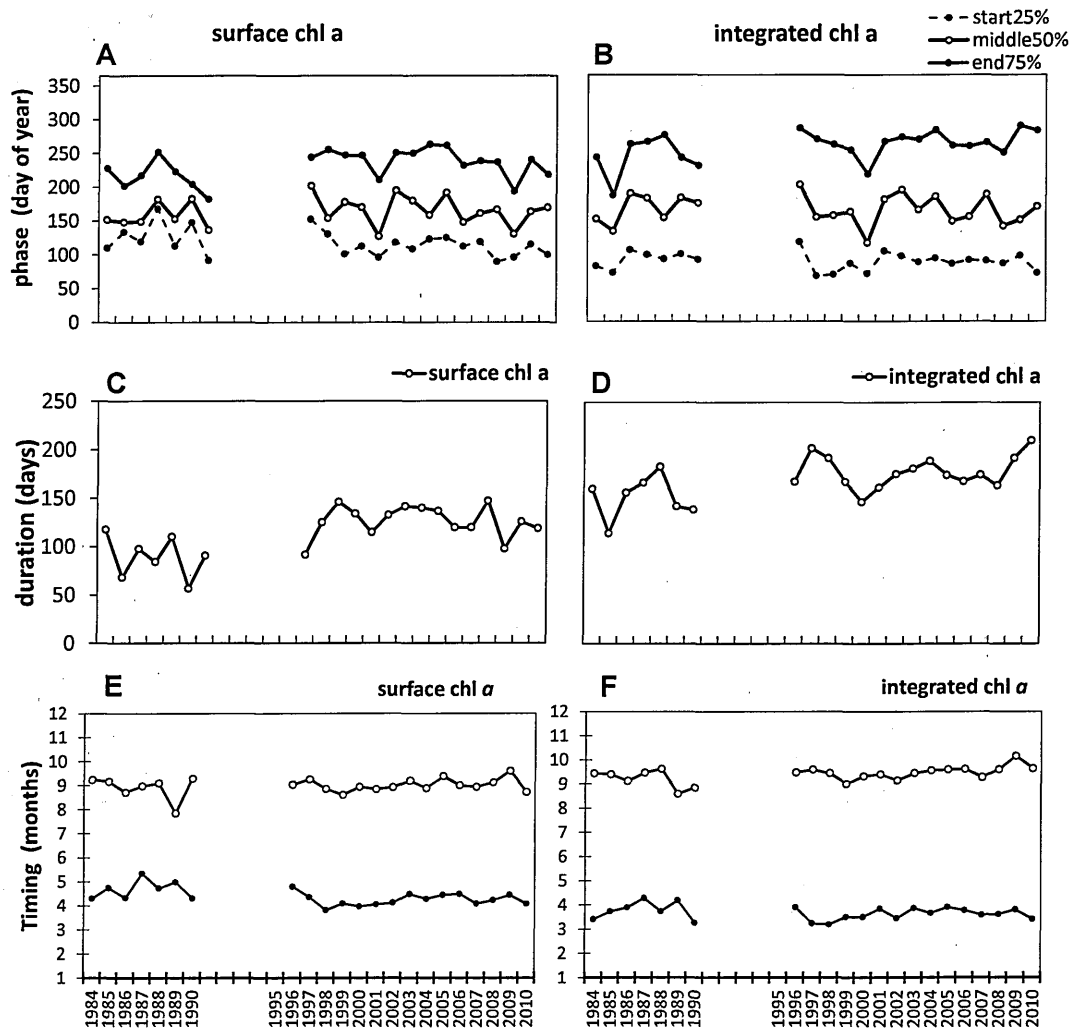

Figure 3.27 Chlorophyll a (chl *a*) phenological events; surface and integrated (A, B) phenophases, (C, D) duration, and (E, F) timing of central tendency from 1984 to 2010. Phenological event and units are indicated on y axes with corresponding units set to same scale for each event. Individual year events are indicated with either circle markers. Note that data from 1995 has been excluded from consideration due to missing months between January and June.

3.5. Comparison between phyto- and zooplankton phenological changes

Phyto- and zooplankton taxa with significant changes in their timing of central tendency (§3.3.2., Table 3.6) were combined for a comparative view of long-term phenological changes in the two compartments. Eight phytoplankton taxa were grouped into 4 functional groups; diatoms (n=5), dinoflagellates (n=1), coccolithophores (n=1), and flagellates (n=1), and 16 zooplankton taxa were grouped into 3 functional groups; copepods (n=10), other holozooplankton (n=4), and meroplankton (n=2) (Table 3.7). The change in timing over three time periods (A) 1984 to 2010, (B) 1984 to 1990, (C) 1995 to 2010, was represented as a function of the timing at the start of each period (Figure 3.28).

Table 3.7 Phytoplankton and zooplankton taxa categories and their reference codes used in Figure 3.28.

category	taxa	code	timing in 1984	timing in 1990	timing in 1995	timing in 2010	2010-1984	1990-1984	2010-1995
diatoms	*Chaetoceros socialis*	Cs	4.3	1.0	3.4	8.7	4.4	-3.3	5.3
diatoms	*Chaetoceros tenuissimus*	Ct	9.5	9.9	8.4	7.6	-1.9	0.3	-0.8
diatoms	*Minidiscus comicus*	Mc	na	7.4	10.1	2.8	2.8	7.4	-7.2
diatoms	*Pseudo-nitzschia multistriata*	Pm	na	na	10.3	7.5	7.5	0.0	-2.8
diatoms	*Skeletonema menzelii*	Sm	na	10.0	11.0	8.0	8.0	na	-3.0
diatoms	*Thalassionema nitzschioides*	Tn	6.2	10.3	6.9	8.1	1.9	4.1	1.2
diatoms	*Thalassiosira mediterranea*	Tm	4.3	2.1	9.2	2.7	-1.7	-2.2	-6.5
diatoms	*Thalassiosira* spp.	T	5.2	3.3	4.0	4.9	-0.3	-1.9	0.9
dinoflagellate	*Heterocapsa niei*	Hn	na	6.2	6.6	5.0	5.0	6.2	-1.6
dinoflagellates	Naked dinoflagellates > 15 µm	d15	10.1	8.0	8.1	7.7	-2.4	-2.1	-0.4
flagellates	Undetermined phytoflagellates >10µm	p10	4.0	5.8	3.1	5.1	1.1	1.8	2.0
coccolithophores	*Emiliana huxleyi*	Eh	9.1	10.3	10.2	8.5	-0.6	1.2	-1.7
copepods	*Isias clavipes*	Ic	9.0	4.9	5.2	4.1	-4.9	-4.1	-1.1
copepods	*Clausocalanus mastigophorus* females	Clm	11.6	9.2	10.7	7.3	-4.3	-2.4	-3.4
copepods	*Clausocalanus paululus* females	Clp	3.1	3.5	4.5	2.8	-0.3	0.4	-1.7
copepods	*Clausocalanus* spp. + *P. Parvus* juveniles	Cl	4.2	3.5	3.7	3.4	-0.8	-0.7	-0.3
copepods	*Farranula rostrata*	Fr	3.1	3.6	4.0	2.8	-0.3	0.5	-1.2
copepods	*Nannocalanus minor*	Nm	9.3	10.2	10.8	8.8	-0.5	0.9	-2.0
copepods	*Oithona setigera* females	Ost	3.3	7.6	9.0	4.3	1.0	4.3	-4.7
copepods	*Oithona similis* females	Osi	4.8	3.2	5.1	3.3	-1.5	-1.6	-1.8
copepods	*Oithona* spp. males juveniles	Oi	4.2	3.1	4.8	3.0	-1.2	-1.1	-1.8
copepods	*Scolecithricella* spp.	Sc	2.4	3.4	na	2.3	-0.1	1.0	2.3
meroplankton	Cirriped larvae	C	3.2	2.6	3.5	3.1	-0.1	-0.6	-0.4
meroplankton	*Pisces* larvae + eggs	Pi	3.1	4.2	3.9	3.5	0.4	1.1	-0.4
other holozooplankton	Appendicularians	A	3.6	2.8	4.4	3.0	-0.6	-0.8	-1.4
other holozooplankton	Ostracods	O	2.6	3.7	4.1	2.1	-0.5	1.1	-2.0
other holozooplankton	*Podon* + *Pleopis*	P	9.0	4.9	6.3	3.5	-5.5	-4.1	-2.8
other holozooplankton	Salps	S	2.1	7.6	8.6	8.8	6.7	5.5	0.2

* Pm timing is reported relative to 1996 as starting point (exludes 1995)

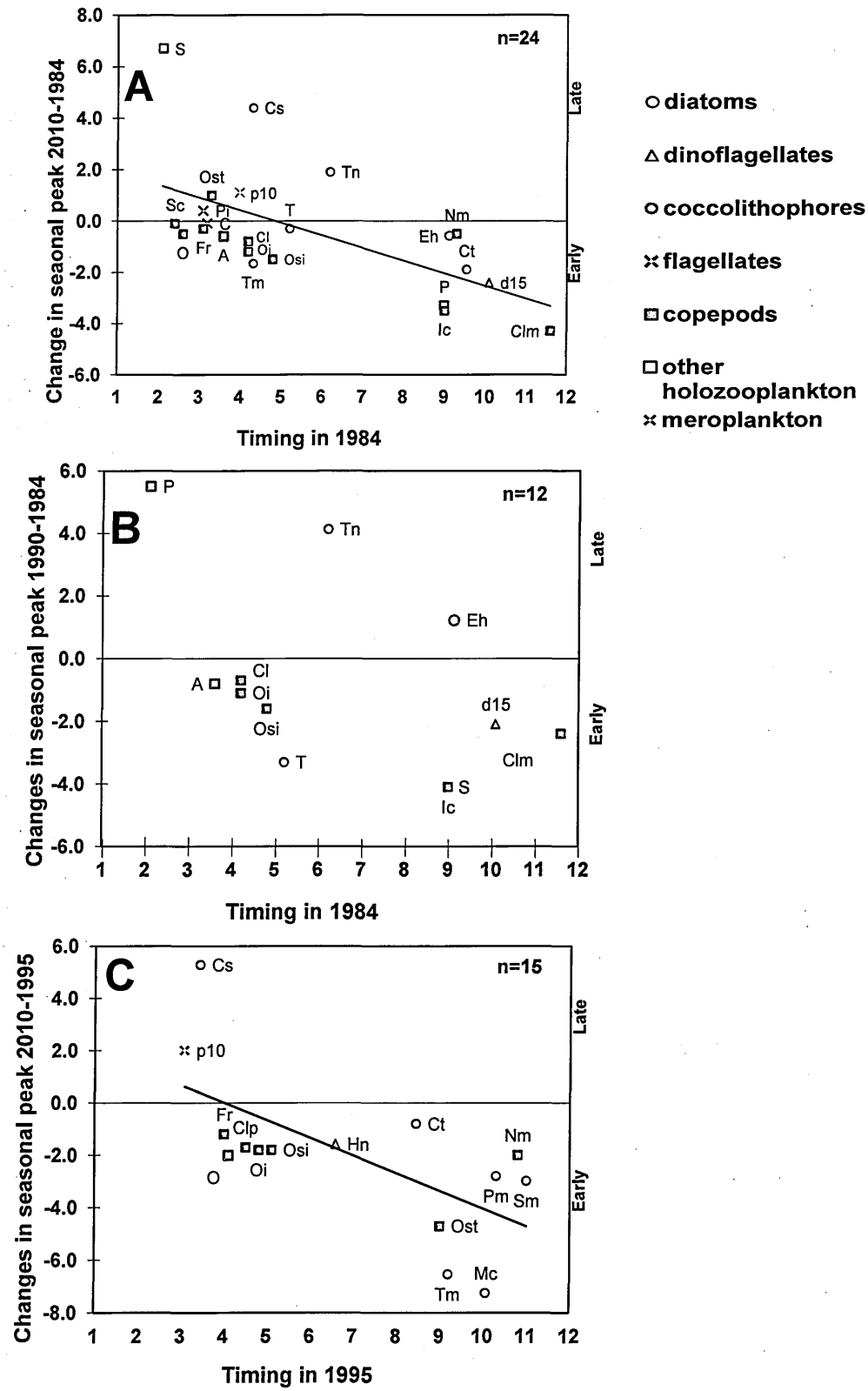

Figure 3.28 Change in timing of central tendency for 24 plankton taxa (A) over 23 year period (2010-1984), (B) part I (1990-1984), and (C) part II (2010-1995). Note: for the diatom species *Pseudo-nitzschia multistriata* (Pm) the change was considered as a function of timing in year 1996 (C).

The comparison between 2010 and 1984 highlighted a significant anticipation of the timing at the end of the 23-years period (n=24 taxa, r^2=0.36, p=0.0018) (Fig. 3.28A). Among phytoplankton only two diatom species were significantly later – *Chaetoceros socialis* (Cs) and *Thalassionema nitzschioides* (Tn), and among zooplankton only salps and the copepod *O. setigera* (Civitarese *et al.*, 1996). Between February and early June, all copepods (excluding Oset) showed a negative trend in timing with changes over 23 years not more than 2 months difference. In contrast, the meroplanktonic group of cirriped larvae (Clv) the copepod *Farranula rostrata* (Fr), and genus *Scolecithricella* spp. (Sc) and Ostracods (O) showed negligible change in their timing between March and April (Fig.3.28 A).

Summer plankton taxa that occurred in July and August did not show a long-term change in timing. However, among taxa that occurred in late summer and autumn, the coccolithophore *Emiliania huxleyi* (Ehux) showed a slightly earlier timing (Fig. 3.28A). *Clausocalanus mastigophorus* (Clmast) females were more than 4 months earlier in their timing between 1984 and 2010 (Fig. 3.28A).

Overall, the difference in timing over a 23 year period showed a consistent earlier pattern in spring and late summer in most phytoplankton and zooplankton taxa. No pattern of change emerged from the difference in timing between 1990 and 1984 (n=12 plankton taxa, n=7 years, r^2=0.23, p=0.1173). Both *T. nitzschioides* (Tn) and salps showed a later timing in 1990 relative to 1984 similar to the long-term trend (Fig. 3.28B). The coccolithophore *E. huxlyei* was slightly more than 1 month later (1.2) in agreement with a significantly later trend in part I of the time series (Table 3.6B). Both the cladoceran *Podon* and the copepod *Isias clavipes* (Iclav) had overlapping timing that was over 4 months earlier in the first period (Fig. 3.26F). The five copepod species that changed timing in the first part of the time series showed a negative change. Overall, the pattern in the first

period was earlier, however there was no significant trend as a result of only few taxa that changed phenology in the first part of the time series.

In the second part of the time series, plankton taxa had a significantly earlier trend in timing between 1995 and 2010 (n=15 taxa, r^2=0.44, p=0.0069) (Fig 3.28C). Most categories were earlier and ranged from between 1 to 2 months earlier between 1995 and 2010 to as much as 6.5 months in the case of *T. mediterranea* (Tm), and 7.3 for *Minidiscus comicus* (Mc). In the case of *M. comicus*, the large change in timing was consistent with the identified pattern which showed a significant change in timing from October to February (Fig. 3.24). Only undetermined phytoflagellates >10 μm and the diatom *Chaetoceros socialis* (Cs) were later in the first half of the year. Diatom species with timing between August and November in 1995 were consistently earlier in 2010, with an anticipation of -2.8 to 3 months for *Pseudo-nitzschia multistriata* (Pm) and *S. menzelii* (Sm), and up to 6-7 months for Tm and Mc. Overall, the 15 plankton taxa that showed consistently earlier trends in timing between 1995 and 2010 shaped the long-term pattern of change in the plankton community.

3.6. Chapter summary

In summary, the following points were revealed by the comparison of long-term changes in the phenology of phyto and zooplankton taxa.

§3.1-3.2

- Seasonal peak abundance varied between both parts of the time series in either magnitude or month of peak abundance. Bulk groups (eg. total phytoplankton, and total cladocerans) with the exception of coccolithophores and gastropods generally maintained the same month of peak abundance. More noticeable shifts in the timing of peak abundance occurred in rare or less abundant taxa and groups.
- Total diatoms acquired a second peak of abundance in August in the second part of the time series which significantly contributed to total phytoplankton abundance.
- Numerous phytoplankton taxa were characterized by bimodal patterns of seasonality and phenology in contrast to zooplankton which exhibited more uniform seasonal peak cycles.

§3.3

- On average between 10 and 20% of the 192 plankton categories showed change in long-term phenological events.
- The timing of central tendency prevailed as the most robust method to determine the phenological events in both phytoplankton and zooplankton with more than 1 seasonal peak.
- Total copepods as a group, and individual species with timing in the first half of the year were significantly earlier: eg. Calanidae juveniles, *Clausocalanus* spp. (males), *Oithona* spp. Males + juveniles, and *Scolecithricella* spp..

§3.4

- Integrated chlorophyll *a* phenology did not reveal substantial long-term change, however there was a general trend towards a longer duration.
- Missing chl *a* measurements in the first half of year 1995 impacted the interpretation of change in the second half of the time series.

§3.5

- Integrated analysis of phytoplankton and zooplankton revealed similar and significant patterns of early timing were observed between spring taxa in the second period and in the whole time series.
- The females of *Clausocalanus mastigophorus* and *Clausocalanus paululus* and males and juveniles of *Clausocalanus* spp. + *P. parvus* showed an earlier change in timing.
- Diatom taxa that occurred between August and November (*C. tenuissimus, T. mediterranea, M. comicus, P. multistriata, S. menzelii*) showed a change in earlier timing in the second part of the time series with the exception of *C. socialis* which showed a much later change in timing relative to 1995.
- Plankton taxa with timing in summer did not change phenology and were missing from comparative analysis of plankton timing.
- Integrated change in plankton phenology was based on a difference relative to one time point and may inaccurately represent long-term change due to variability the occurrence of planktonic organisms.

The possible mechanisms driving the long-term changes in phenology (identified in Chapter 3) will be explored in Chapter 4 in relation to environmental parameters and later discussed in Chapter 5.

Chapter 4

RELATIONSHIPS BETWEEN CHANGES IN PLANKTON PHENOLOGY AND ENVIRONMENTAL PARAMETERS

4.1. Seasonal cycle and interannual anomalies of environmental parameters at LTER-MC

To unveil the role of environmental parameters as possible drivers of plankton phenology, the phenological events that showed significant changes at LTER-MC (identified in Chapter 3) were analyzed in relation to seasonal and monthly anomalies of surface and integrated temperature, salinity and chlorophyll *a* (chl *a*). The seasonal patterns and the interannual anomalies of the environmental parameters are herein described before the results of correlation analysis.

The mean seasonal cycle of surface temperature (2 m) revealed a clear seasonal signature (Fig. 4.1A). The lowest values recorded in winter ranged from 14.10±0.48 °C to 14.25±0.48 °C in February-March; a gradual increase occurred from spring to summer until a peak of 25.91±1.51 °C in August, which was followed by a decrease to 17.07±0.69 °C in December. Depth integrated temperature (0-70 m) showed a smoother seasonal cycle with a similar winter minimum of 14.08±0.56 °C and a peak of 18.93±0.89 °C in October. (Fig. 4.1B).

Seasonal surface salinity ranged from 37.30±0.28 in May to 37.89±0.17 in October, with a minimum in May (Fig. 4.1C). The seasonal variability was less pronounced in the depth-integrated salinity, which also showed the lowest value in May (37.79±0.14 psu) (Fig. 4.1D).

The annual cycle of chl *a* showed the lowest concentations always in January and December, both at the surface (0.50±0.28 μg L^{-1}) and in the whole water column (0.36±0.18 μg L^{-1}) (Figs. 4.1 E, F). The major peak was recorded in May at the surface (2.88±6.57 μg L^{-1}, Fig.4.1E) and in March in the integrated water column values (0.80±0.54 μg L^{-1}) (Fig. 4.1F). A secondary increase of chl *a* concentration was observed in October both at the surface concentrations (0.59±0.23 μg L^{-1}) and when chl a values were integrated over the water column (0.59±0.23 μg l^{-1}).

The annual anomalies of temperature in winter showed an almost regular three year-alternation of negative and positive anomalies until 2007 when the highest positive anomaly was recorded (Fig.4.2A, B). In January 2007 the mean integrated temperature was 16.26±0.43 °C, i.e about 1 °C higher than the mean (15.22±0.52 °C). In the same year, surface temperatures were also higher in February and March, ranging from 15.24 °C to 15.09 °C respectively, nearly more than 1 °C above the general mean. In spring, surface temperature anomalies shifted from negative to positive values in 1996 at the surface (Fig.4.2C), and in 1998 in the water column (Fig.4.2D). Anomalies of depth integrated temperatures in spring significantly increased in the first part of the time series in March and April (Table 4.1B). In summer, the temperature anomalies changed from prevalent negative values in the first part of the time series to prevalent positive values in the second part (Fig.4.2E, F). There was a significant positive trend ($p=0.0370$) in surface summer temperature anomalies in the second part of the time series (Table 4.1A). In autumn, the interannual pattern of temperature anomalies was more variable, with the exception of a continuous period of positive anomalies from 1999 to 2006 at the surface (Fig. 4.2 G) and from 2006 to 2011 in the integrated water column (Fig 4.2H).

The inter-annual patterns of salinity in all seasons showed a switch from negative to positive anomalies in 1987, which lasted until 1995 (Fig. 4.3 A-H). An increasing pattern of negative anomalies was observed in spring surface salinity from 2004 to 2010 (Fig.4.3C). In autumn, a regular alternation of 3-4 years of positive and negative anomalies was recorded both at the surface and in the water column (Fig.4.3 G, H.).

The inter-annual variability of chl *a* showed a decreasing pattern from high positive anomalies in the first part of the time series to negative anomalies in the second part of the time series (Fig.4.4 A-H). This pattern was observed in all seasons. It was interrupted only in sparse years of the second part of the time series that showed low positive anomalies, the most relevant ones in the water column in autumn (Fig. 4.4H). In particular, the highest

surface chl *a* anomalies were mostly recorded in the earliest years of the time series between 1984 and 1990.

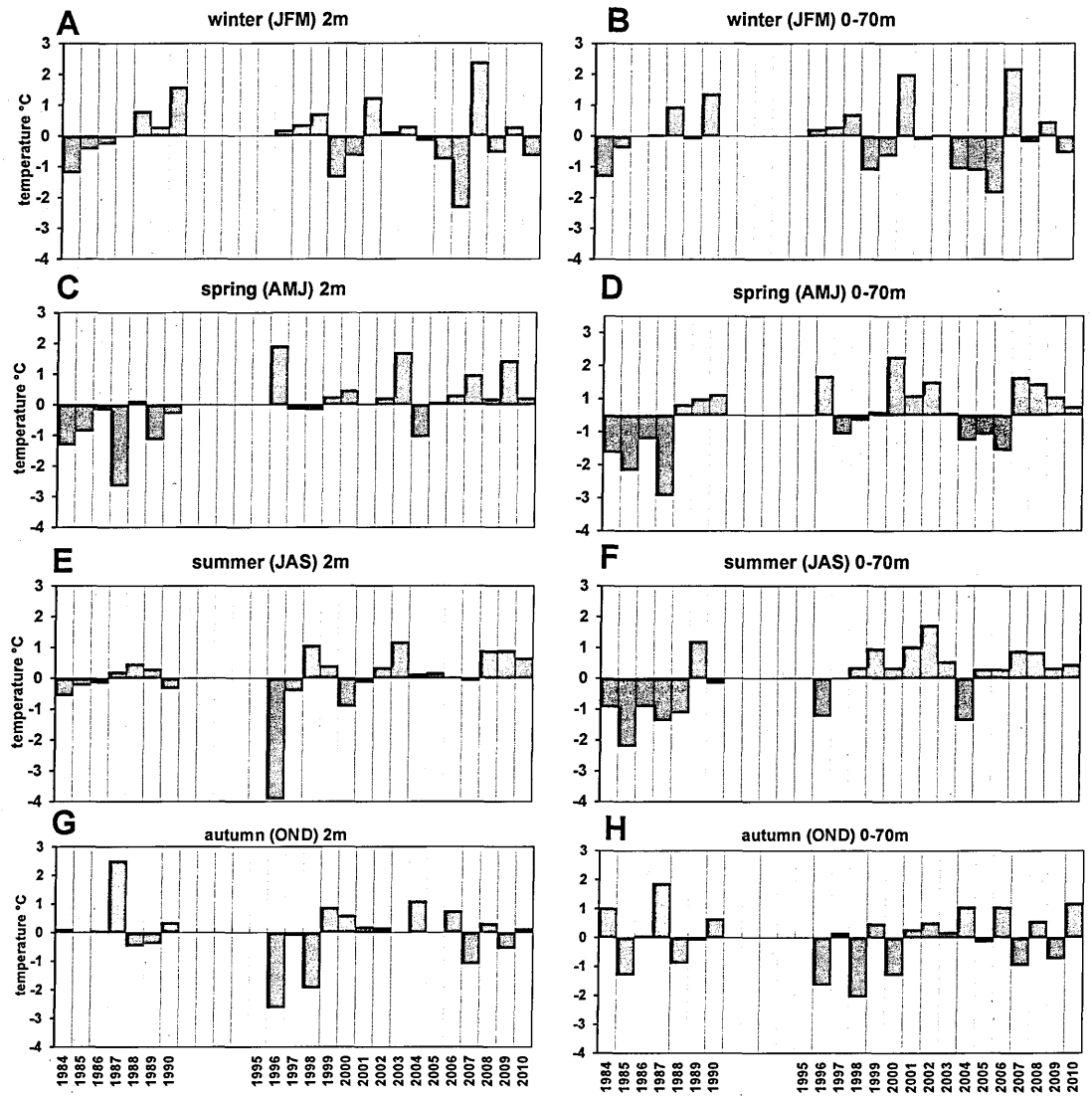

Figure 4.2 Inter-annual temperature anomalies at surface (2m) and integrated (0-70m) depth; (A, B) winter anomalies consider months (January-March, JFM), (C,D) spring anomalies (April-June, AMJ), E, F), summer anomalies (July-September, JAS), (G, H) autumn anomalies (October-December, OND).

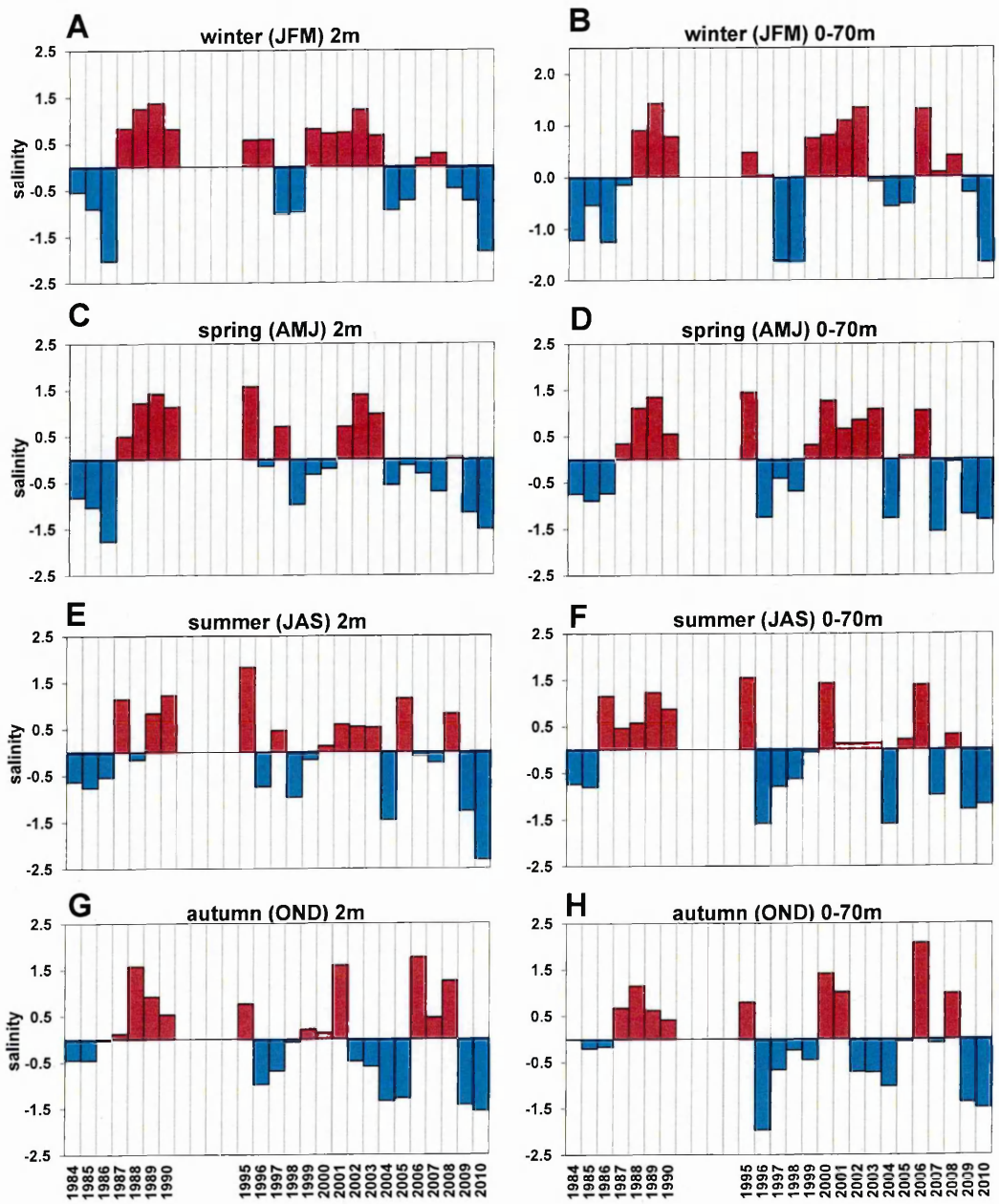

Figure 4.3 Inter-annual salinity anomalies at surface (2m) and integrated (0-70m) depth; (A, B) winter anomalies consider months (January-March, JFM), (C,D) spring anomalies (April-June, AMJ), E, F), summer anomalies (July-September, JAS), (G, H) autumn anomalies (October-December, OND).

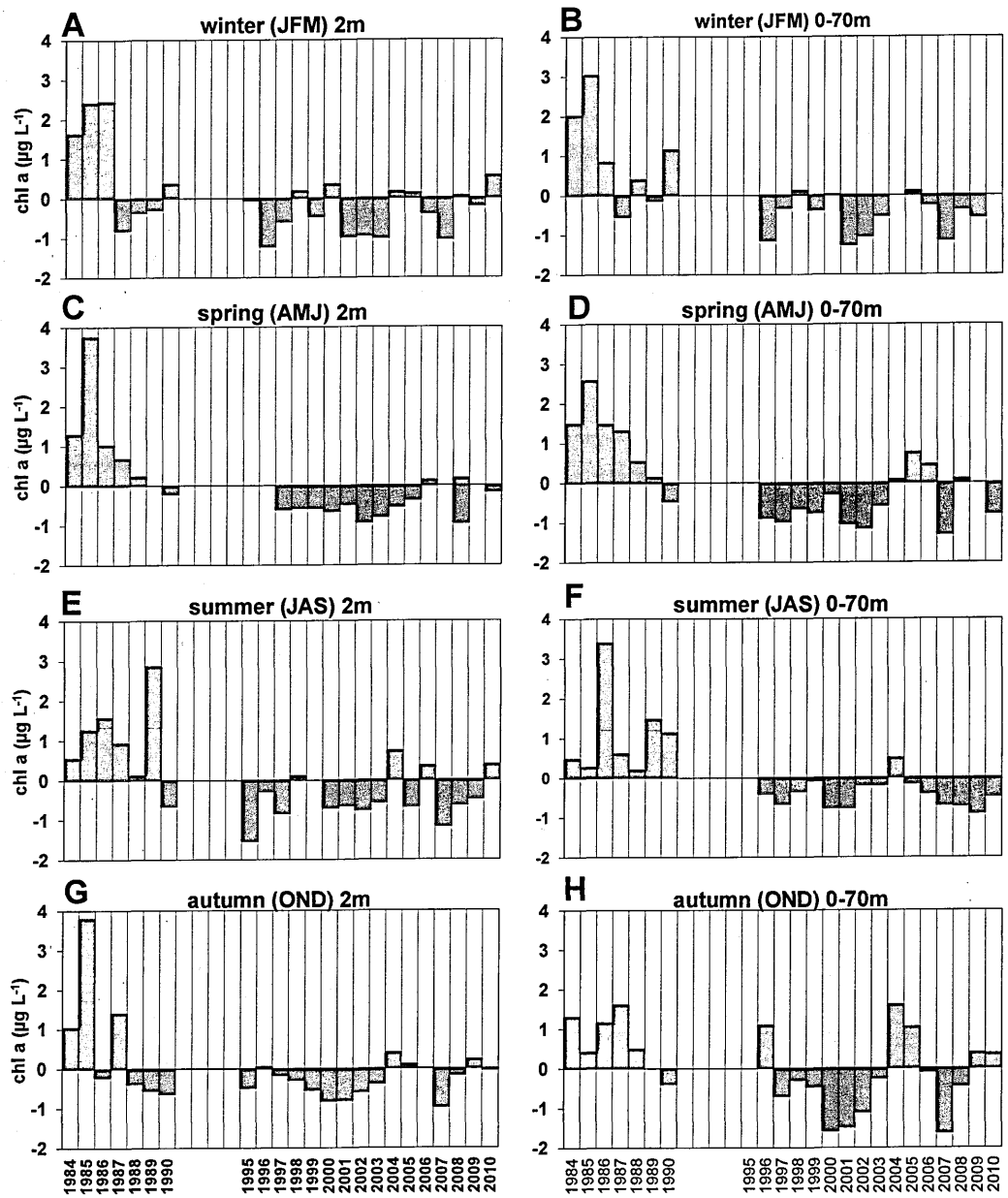

Figure 4.4 Inter-annual chlorophyll (chl *a*)anomalies at surface (2m) and integrated (0-70m) depth; (A, B) winter anomalies consider months (January-March, JFM), (C,D) spring anomalies (April-June, AMJ), E, F), summer anomalies (July-September, JAS), (G, H) autumn anomalies (October-December, OND).

Table 4.1 Trends in monthly temperature (A, B) and salinity anomalies (C, D) at surface (2 m, A, C) and integrated (0-70 m, B, D) depth within the first (1984-1990) and second part (1995-2010) of the time series. Significant linear trends and corresponding p-values for each month are bold highlighted. General mean temperature values (°C), and salinity values are reported for the whole time series (1984-2010). The trend is indicated as increasing or decreasing with red ↑ or blue ↓ arrows, respectively.

A

Surface Temp anomalies	1984-1990 p-value	1995-2010 p-value	trend	monthly mean temp °C 1984-1990	1995-2010
JANUARY	0.1287	0.7333	n.s.	15.20	15.10
FEBRUARY	0.0895	0.6627	n.s.	14.32	14.23
MARCH	**0.0106**	0.8741	↑	**14.10**	14.09
APRIL	0.2190	0.4369	n.s.	15.17	15.89
MAY	0.8003	0.9762	n.s.	18.47	19.91
JUNE	0.9599	0.9052	n.s.	21.77	23.45
JULY	0.5935	**0.0370**	↑	24.77	**25.48**
AUGUST	0.2932	0.0833	n.s.	26.25	25.77
SEPTEMBER	0.8841	0.1546	n.s.	23.97	23.98
OCTOBER	0.8010	0.2140	n.s.	22.03	21.48
NOVEMBER	0.5959	0.1039	n.s.	19.32	19.12
DECEMBER	0.2033	0.9651	n.s.	17.21	17.00

B

Integrated Temp anomalies	1984-1990 p-value	1995-2010 p-value	trend	monthly mean temp °C 1984-1990	1995-2010
JANUARY	0.2323	0.8713	n.s.	15.32	15.17
FEBRUARY	0.1030	0.6516	n.s.	14.37	14.31
MARCH	**0.0072**	0.7735	↑	**14.05**	14.09
APRIL	**0.0305**	0.5570	↑	**14.65**	14.74
MAY	0.2057	0.9995	n.s.	15.38	16.09
JUNE	0.1616	0.6660	n.s.	16.46	17.46
JULY	0.2749	0.6639	n.s.	17.37	18.01
AUGUST	0.0694	0.9859	n.s.	17.90	18.73
SEPTEMBER	0.2975	0.1372	n.s.	18.60	19.08
OCTOBER	0.2941	0.0664	n.s.	19.12	18.85
NOVEMBER	0.7708	0.2355	n.s.	18.54	18.54
DECEMBER	0.1805	0.8288	n.s.	17.21	17.06

C

Surface salinity anomalies	1984-1990 p-value	1995-2010 p-value	trend	monthly mean salinity 1984-1990	1995-2010
JANUARY	0.2852	0.2483	n.s.	37.92	37.86
FEBRUARY	0.0525	0.7642	n.s.	37.87	37.83
MARCH	0.2304	**0.0109**	↓	37.60	**37.61**
APRIL	0.0689	**0.0091**	↓	37.53	**37.50**
MAY	0.1464	0.5679	n.s.	37.32	37.29
JUNE	0.4892	**0.0013**	↓	37.41	**37.38**
JULY	0.3304	**0.0265**	↓	37.62	**37.61**
AUGUST	0.0910	0.1672	n.s.	37.88	37.79
SEPTEMBER	0.6001	0.5456	n.s.	37.90	37.89
OCTOBER	0.1691	0.9949	n.s.	37.96	37.85
NOVEMBER	0.1632	0.3364	n.s.	37.88	37.84
DECEMBER	0.5668	0.4116	n.s.	37.91	37.86

D

Integrated monthly anomalies	1984-1990 p-value	1995-2010 p-value	trend	monthly mean salinity 1984-1990	1995-2010
JANUARY	0.0815	0.6203	n.s.	37.99	37.94
FEBRUARY	**0.0316**	0.9496	↑	**37.89**	37.93
MARCH	0.1731	0.1680	n.s.	37.89	37.89
APRIL	0.1247	0.2625	n.s.	37.89	37.85
MAY	0.0657	0.3224	n.s.	37.78	37.80
JUNE	**0.0413**	0.1061	↑	**37.86**	37.80
JULY	**0.0068**	0.2175	↑	**37.87**	37.85
AUGUST	0.6806	0.3713	n.s.	37.98	37.89
SEPTEMBER	**0.0032**	0.8870	↑	37.98	37.91
OCTOBER	0.1406	0.7784	n.s.	38.00	37.92
NOVEMBER	0.0529	0.7234	n.s.	38.03	37.94
DECEMBER	0.7008	0.9529	n.s.	37.97	37.94

In summary, the annual anomalies showed a significant increasing trend in surface temperature in summer (July) and a decreasing trend in salinity in late winter to spring (March and April) and summer (June and July). In the first part of the time series, temperature anomalies in winter and spring generally followed the same pattern as salinity anomalies with a switch from negative anomalies that occurred between 1984 and 1986 to positive anomalies starting in 1987 in the case of salinity followed by positive years of temperature starting in 1988. In contrast, chlorophyll anomalies switched from positive to negative anomalies between the two periods of the time series. The selected environmental parameters show some significant long-term trends in the seasonal and monthly patterns (Table 4.1). Temperature, salinity and chlorophyll appear to have corresponding patterns which will be considered in light of their correlation with long-term plankton phenology. Temperature and salinity appear to follow a similar pattern in the first period of the time series (Fig 4.2 and 4.3) whereas chl *a* displayed an inverse pattern (Fig. 4.4). The relationship between higher temperatures and salinity and corresponding lower chlorophyll will be discussed in detail in Chapter 5.

Plankton phenological events that showed significant changes at LTER-MC, as identified and described in Chapter 3, were analyzed in relation to selected environmental parameters to investigate the dependence of phenological changes on the water column characteristics. Phenological events such as phenophases, duration and timing of central tendency in phyto- and zooplankton were correlated with monthly and/or seasonal anomalies of temperature, salinity and as presented in the following sections.

4.2.1 Correlations of phytoplankton phenology with temperature and salinity anomalies

Significant correlations between phytoplankton phenological events and monthly or seasonal anomalies of temperature and salinity were obtained for 13 phytoplankton taxa including 8 categories of diatoms, 2 dinoflagellates, 2 other flagellates and total coccolithophores (Table 4.2). Correlations with seasonal anomalies are presented for surface parameters only, while correlations with monthly anomalies are presented for both surface and depth integrated parameters.

Table 4.2 Significant correlations between phytoplankton phenological events and anomalies of surface and depth integrated temperature and salinity.Pairwise correlation coefficients (*r)* and significance at * ($p \leq 0.05$).. Seasonal anomalies ($^{\pm}$) are considered JFM as winter, AMJ as spring, JAS as summer, OND as summer. Categories with >1 correlation are indicated with a quote (").

group	category	phenology event	parameter	anomalies	*r*
diatom	pennate diatoms >10 µm	duration	temp surface	August	0.44 *
	Chaetoceros affinis	duration	salinity surface	JFM$^{\pm}$	0.47 *
	"	duration	salinity integrated	February	0.54 *
	"	duration	salinity integrated	March	0.50 *
	"	duration	temp integrated	April	0.47 *
	Chaetoceros anastomatus	duration	temp surface	August	0.47 *
	Chaetoceros socialis	phenophases (start)	salinity surface	June	-0.49 *
	Chaetoceros tenuissimus	phenophases (start)	salinity surface	AMJ±	-0.55 **
	"	phenophases (start)	salinity surface	March	-0.52 *
	"	phenophases (start)	salinity surface	April	-0.57 **
	"	timing [7:12]	temp surface	JAS$^{\pm}$	-0.47 *
	"	timing [1:6]	temp integrated	May	0.44 *
	Minidiscus comicus	timing [1:12]	salinity surface	AMJ±	0.62 *
	"	timing [1:12]	salinity surface	June	0.45 *
	Thalassiosira mediterranea	timing [1:12]	temp surface	AMJ$^{\pm}$	-0.42 *
	"	duration	salinity surface	March	0.50 *
	Thalassiosira spp.	duration	salinity surface	JAS±	0.46 *
	"	duration	temp surface	March	0.47 *
	"	timing [1:6]	temp integrated	June	-0.44 *
dinoflagellate	*Heterocapsa niei*	timing [1:12]	salinity surface	AMJ±	0.44 *
	"	timing [1:12]	temp surface	May	0.43 *
	"	phenophases (start)	salinity surface	March	0.46 *
	"	phenophases (start)	salinity surface	April	0.46 *
	Naked dinoflagellates >15µm	timing [7:12]	salinity surface	August	0.47 *
other flagellate	undetermined phytoflagellates>10µm	timing [1:6]	temp integrated	April	-0.42 *
	Dinobryon faculiferum	phenophases (start)	salinity surface	June	-0.62 **
	"	phenophases (start)	salinity integrated	June	-0.52 *
	"	phenophases (start)	salinity surface	July	-0.52 *
	"	phenophases (start)	salinity integrated	July	-0.53 **
coccolithophore	total coccolithophores	timing [7:12]	salinity surface	September	-0.50 *

$^{\pm}$ seasonal anomalies (JFM, AMJ, JAS, OND)

4.2.1 Phytoplankton phenology and temperature anomalies

The timing of central tendency of the diatom species *C. tenuissimus* and *T. mediterranea,* was negatively correlated with summer and spring temperature anomalies, respectively. The delayed timing of *C. tenuissimus*, a species with bimodal seasonality was negatively correlated in the first half of the year with integrated temperature in May (Table 4.2). In the second part of the year *C. tenuissimus* showed a reccurent timing in August for 11 out of 23 years; however the timing occurred later in years when summer temperature anomalies were negative (Fig. 4.5). In particular, the bimodal pattern of timing was later in the first part of the time series (1984, 1985 and 1990).

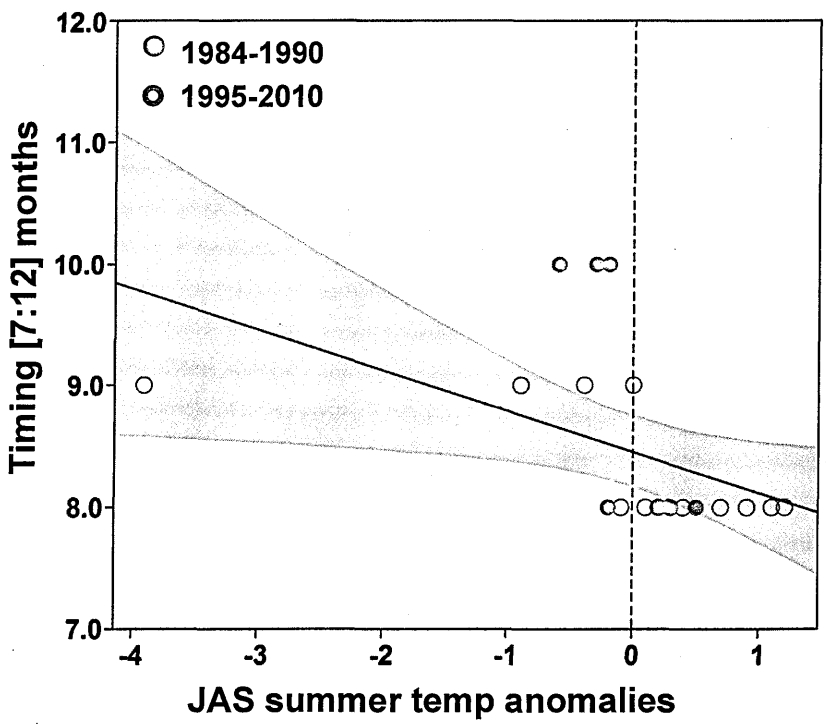

Figure 4.5 Timing of central tendency of *Chaetoceros tenuissimus* as a function of summer (JAS) surface temperature anomalies. Shaded region indicates the 95% confidence area for a bivariate fit of timing by temperature anomalies.

The timing of *T. mediterranea*, a species generally peaking in early spring, was weakly correlated with surface spring temperature anomalies (Fig. 4.6). The timing occurred more frequently in March, with a tendency to occur later (until June) in

correspondence with negative temperature anomalies and earlier (in February and January) in correspondence with positive anomalies. The timing was significantly earlier in the second part of the time series (open circles, Fig. 4.6). It is worth noting that in 1987, the timing occurred in September, and this year of late occurrence likely influenced the significance of the regression. Note that spring surface temperature anomalies were not calculated due to missing data in 1995.

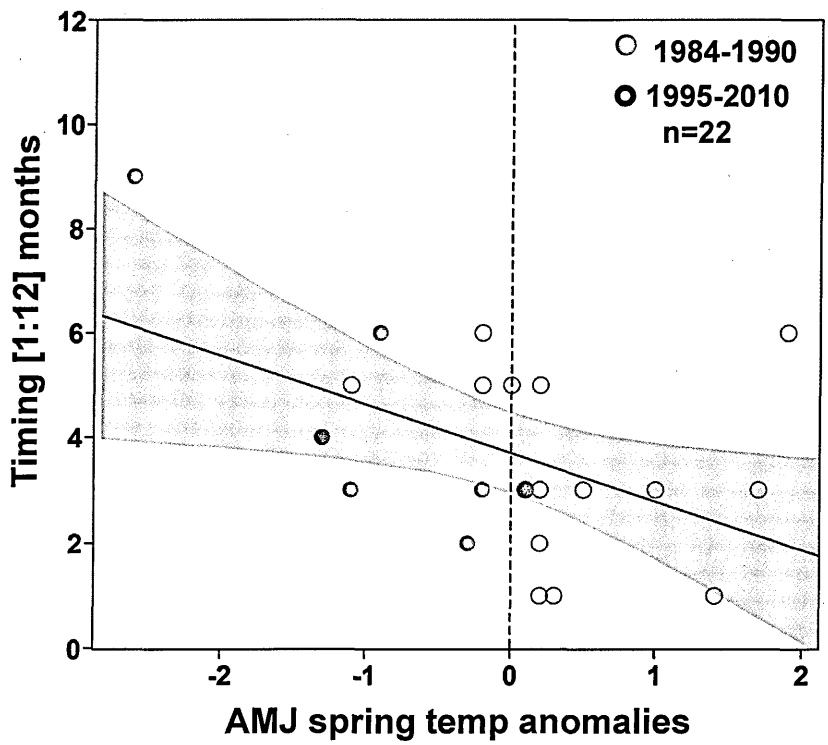

Figure 4.6 Timing of central tendency of *Thalassiosira mediterranea* as a function of spring (AMJ) surface temperature anomalies. Shaded region indicates the 95% confidence area for a bivariate fit of timing by temperature anomalies.

Several other taxa showed significant correlations between phenological events and monthly temperature anomalies (Table 4.2). In diatoms, the genus *Chaetoceros* showed positive trends in the duration of *Chaetoceros affinis* and *C. anastomosans* in relation to monthly temperature anomalies in April and August respectively, indicating that the season was longer when temperature anomalies were positive. In addition to *T. mediterranea*, the duration of *Thalassiosira* spp. was positively correlated with March surface temperature anomalies (Table 4.2). The timing of dinoflagellate *Heterocapsa niei* was positively correlated with May surface anomalies. Two bulk groups also had significant correlations with monthly temperature anomalies. Pennate diatoms >10 μm showed a positive trend of longer duration with August anomalies of surface temperature, and undetermined phytoflagellates >10 μm were negatively correlated with April integrated temperature anomalies indicating their timing occurred earlier in the first half of the year when anomalies were positive (Table 4.2).

4.2.2 Phytoplankton phenology and salinity anomalies

The correlation of phytoplankton phenology with seasonal anomalies of surface salinity was negative for the diatom *C. tenuissimus* and positive for other four taxa, which included three diatoms and a dinoflagellate (Table 4.2). More numerous correlations were found between phenological events and monthly salinity anomalies.

The start phenophase of *C. tenuissimus* was negatively correlated (n=23, p=0.0063) with spring seasonal anomalies of salinity (Fig. 4.7), and March and April monthly surface salinity anomalies (Table 4.2), i.e., the onset of this species occurred earlier in years of positive salinity anomalies.

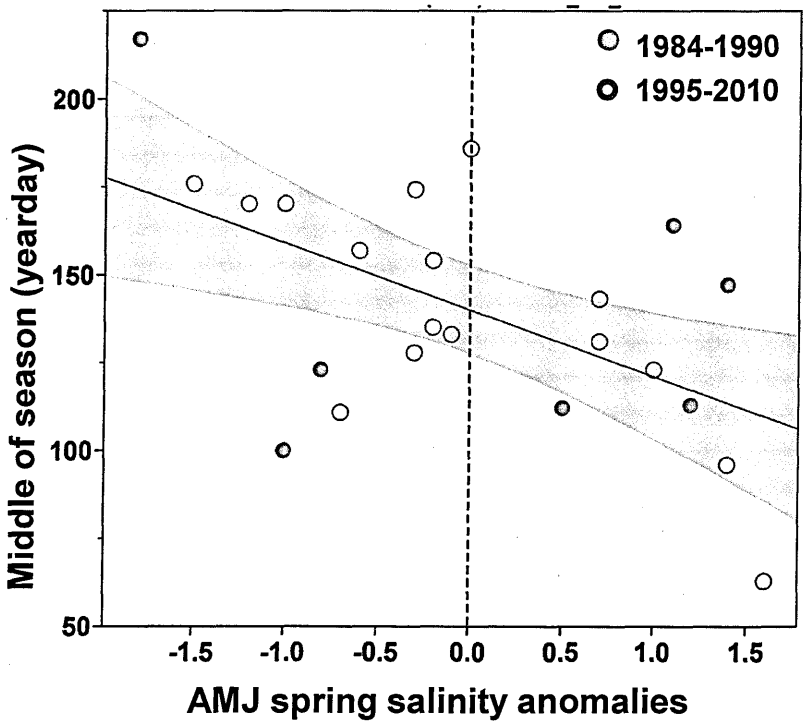

Figure 4.7 Start phenophase of *Chaetoceros tenuissimus* as a function of spring (AMJ) surface salinity anomalies.Shaded region indicates the 95% confidence area for a bivariate fit of phenophase by salinity anomalies.

The duration of *Chaetoceros affinis* occurrence was longer in years with positive surface salinity anomalies in winter (Fig. 4.8), and depth-integrated salinity anomalies in February and March (Table 4.2). Year 1996 was omitted because this taxon had two discrete records of abundance, on 1/30/1996 and 4/10/1996, resulting in a single phenophase calculation of day 69 for start, middle and end phases. Thus, the duration in 1996 was 0 days in length. In years 1998, 2001 and 2004, only the start phenophase was calculated, while in 1999 the species was not recorded.

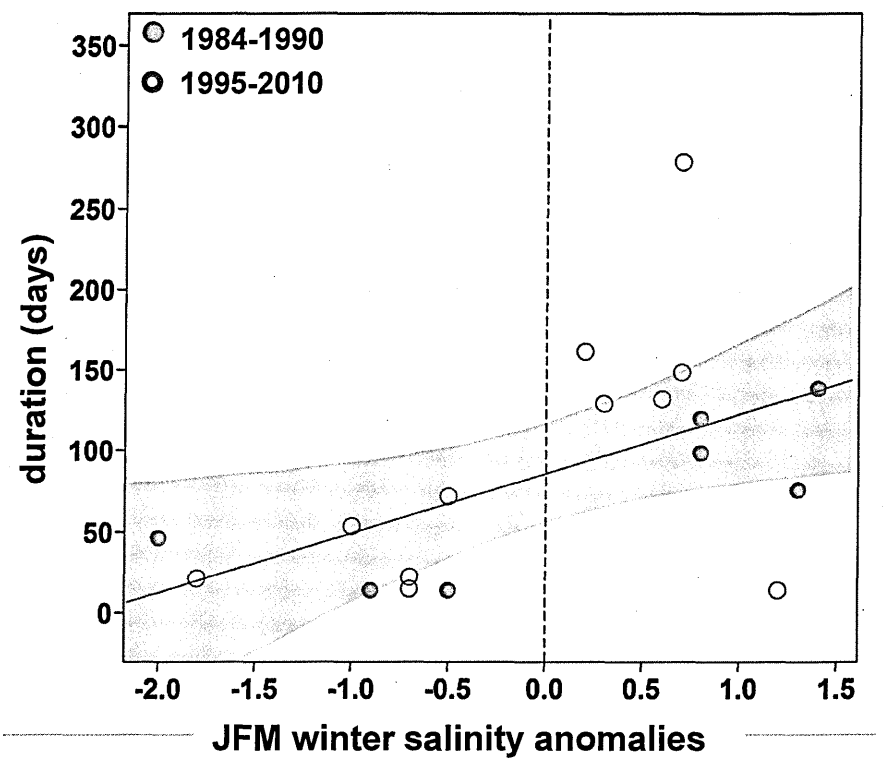

Figure 4.8 Phenological duration of *Chaetoceros affinis* as a function of winter (JFM) surface salinity anomalies. Years 1996, 1998 and 1999, 2001 and 2004 were omitted due to sporadic abundance and missing middle and end phenophase data. Shaded region indicates the 95% confidence area for a bivariate fit of duration by salinity anomalies.

Changes in the timing of central tendency in *M. comicus*, were positively correlated with spring anomalies of surface salinity (Fig. 4.9), which was a reflection of the positive correlation with June surface anomalies (Table 4.2). The timing of this bimodal taxa shifted to March, during years of negative spring anomalies, between 2003 and 2010 with the exception of 2007 when the species was not recorded in counted samples. The unusual phenology of this taxon (also visualized in Fig. 3.17), combined with a shift in phenology from the second half to the early portion of the year, suggests a possible relationship with some years of negative salinity anomalies.

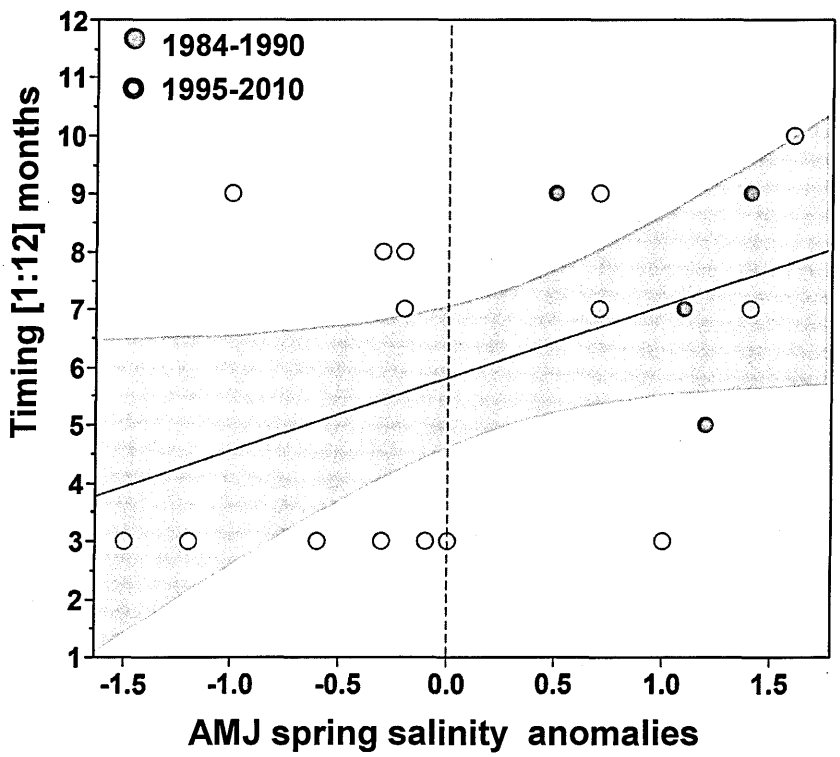

Figure 4.9 Timing of central tendency of *Minidiscus comicus* as a function of spring (AMJ) salinity anomalies. Years 1984,1985, 1986 and 2007 were omitted from regression analysis because the taxa did not occur regularly until 1987, and no abundance was recorded in 2007. Shaded region indicates the 95% confidence area for a bivariate fit of timing by salinity anomalies.

The duration of *Thalassiosira* spp. occurrence was positively correlated (p=0.0287) with higher summer surface salinities (Fig. 4.10). The duration was significantly longer in correspondence of positive anomalies. Despite this significant correlation with seasonal salinity anomalies, there was no relationship between changes in duration for this genus and other species and specific monthly anomalies of salinity.

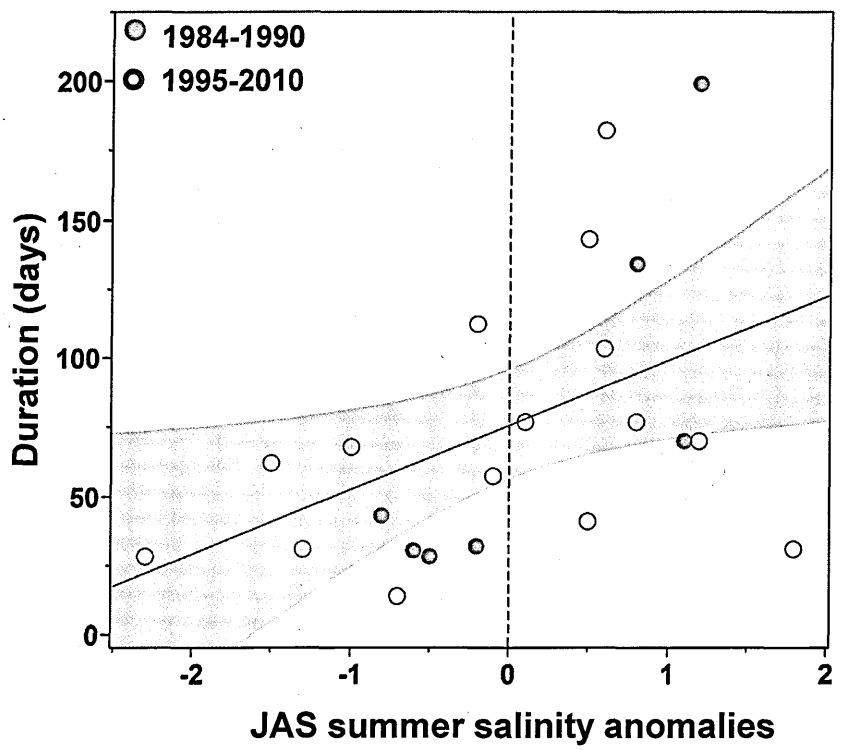

Figure 4.10 Duration of *Thalassiosira* spp. occurrence as a function of summer (JAS) surface salinity anomalies. Shaded region indicates the 95% confidence area for a bivariate fit of duration by salinity anomalies.

The timing of the central tendency in the dinoflagellate species *Heterocapsa niei* was positively correlated with spring salinity anomalies (Fig. 4.11). The timing coincided with May during negative salinity anomalies in 1986 and from 2005 to 2010, and mostly in June and July during positive salinity anomalies. The onset of this taxa (start25% phenophase) was significantly positively correlated with surface salinity anomalies in March and April (Table 4.2).

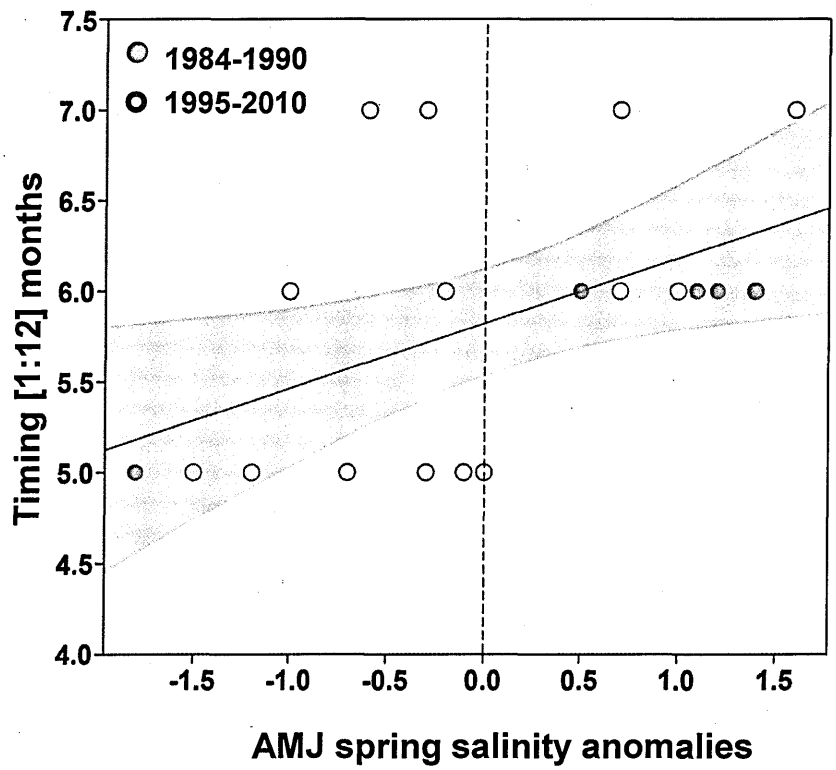

Figure 4.11 Timing of central tendency of *Heterocapsa niei* as a function of spring (AMJ) salinity anomalies. Years 1984 and 1985 were not reported because the species was not recorded in cell counts. Shaded region indicates the 95% confidence area for a bivariate fit of timing by salinity anomalies.

Some phytoplankton categories which showed change in their phenology showed significantly correlations with monthly scale salinity anomalies only. For example, the onset (start phenophase) of the abundant diatom *C. socialis* was significantly earlier and negatively correlated ($p=0.0175$) with June surface salinity anomalies (Table 4.2). The onset of flagellate *D. faculiferum* was significantly earlier in relation to June and July surface and integrated salinity anomalies. Additionally, the timing of naked dinoflagellates >15 μm in the second half of the year was positively correlated with August surface salinity anomalies, whereas the timing of total coccolithophores was significantly ($p=0.0141$) and negatively correlated with September salinity anomalies (Table 4.2).

4.3.1 Correlations of zooplankton phenology with temperature anomalies

Within the zooplankton community, 9 categories showed significant correlations between some phenological indexes and seasonal anomalies of surface or integrated temperature. These categories include total zooplankton, total copepods, three minor groups, and four copepod taxa (Table 4.3). Most correlations were obtained with the timing of central tendency.

Table 4.3 Significant correlations between zooplankton phenological events and seasonal anomalies of surface and depth-integrated temperature anomalies. Zooplankton categories are listed alphabetically by group and by copepod species. Pairwise correlation coefficients (*r)* and significance at * ($p \leq 0.05$).

category	phenology event	seasonal anomalies	depth	*r*
Bivalve larvae	start phase	summer	surface	0.42 *
Cirripede larvae	timing [1:6]	winter	surface	-0.56 **
Cirripede larvae	timing [1:6]	winter	integrated	-0.60 **
Salps	timing [1:12]	summer	integrated	0.59 **
Total copepods	timing [1:6]	summer	integrated	-0.43 *
Total zooplankton	timing [1:6]	summer	integrated	-0.45 *
Clausocalanus spp.	timing [1:6]	spring	surface	-0.48 *
Clausocalanus spp.	timing [1:6]	spring	integrated	-0.58 **
Farranula rostrata	timing [1:6]	spring	integrated	0.43 *
Oithona setigera	timing [1:12]	autumn	integrated	-0.61 **
Temora stylifera	duration	spring	integrated	0.44 *

The timing of total zooplankton and total copepods showed a similar significantly negative correlation with summer anomalies of integrated temperature, i.e. the timing was anticipated in correspondence of positive anomalies (Fig.4.12). The timing of salps was instead correlated positively with summer anomalies of integrated temperature, with later timing in years of positive anomalies (Table 4.3, Fig. 4.13). Among meroplankton, cirriped larvae showed an earlier timing in years of positive winter temperature anomalies (Fig. 4.14).

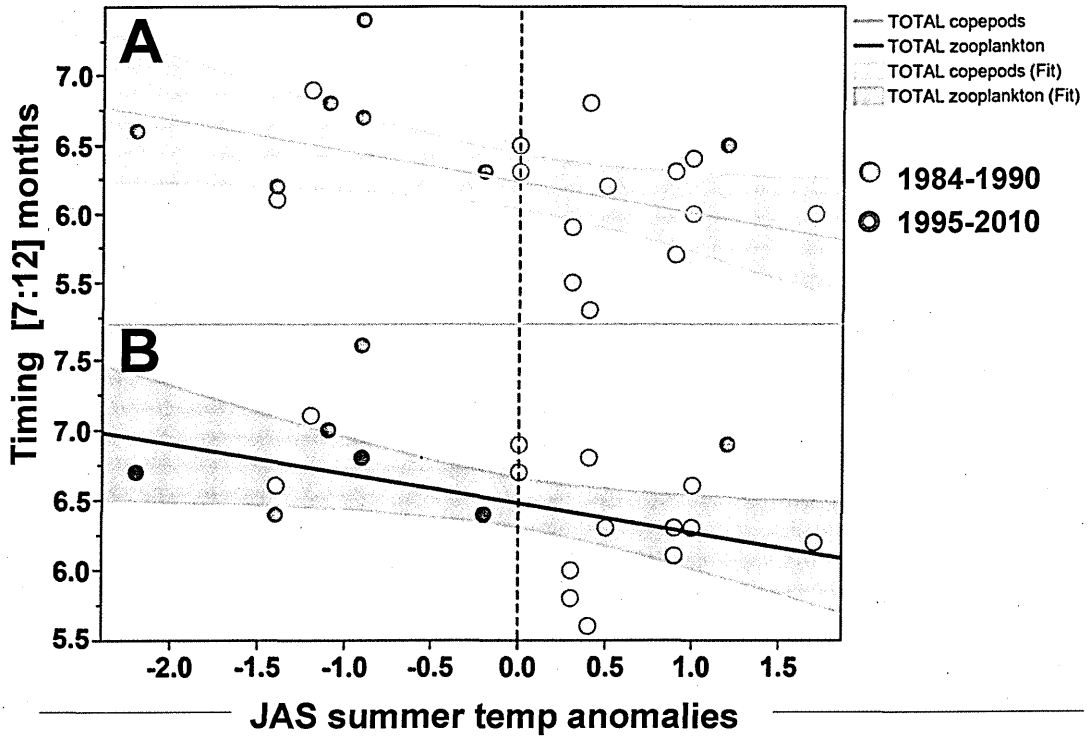

Figure 4.12 Timing of central tendency of (A) total copepods and (B) total zooplankton as a function of summer (JAS) anomalies of depth integrated temperature. Note that due to concurrent timing in years 2006 and 2009, only 14 open circles reflect the second part of the time series.

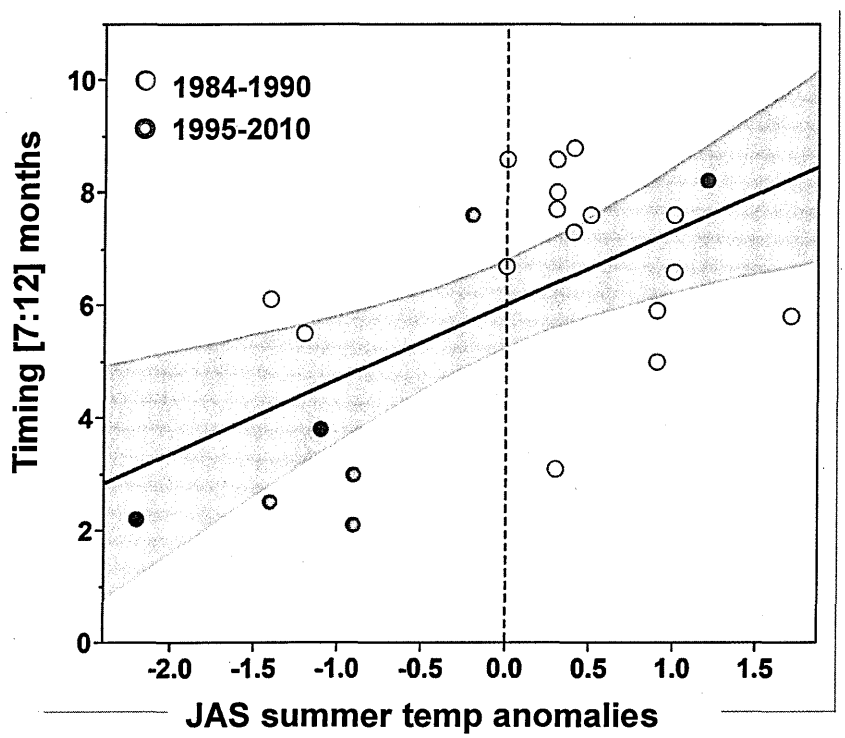

Figure 4.13 Timing of central tendency of salps as a function of summer (JAS) depth integrated temperature anomalies. Shaded region indicates the 95% confidence area for a bivariate fit of phenophase by salinity.

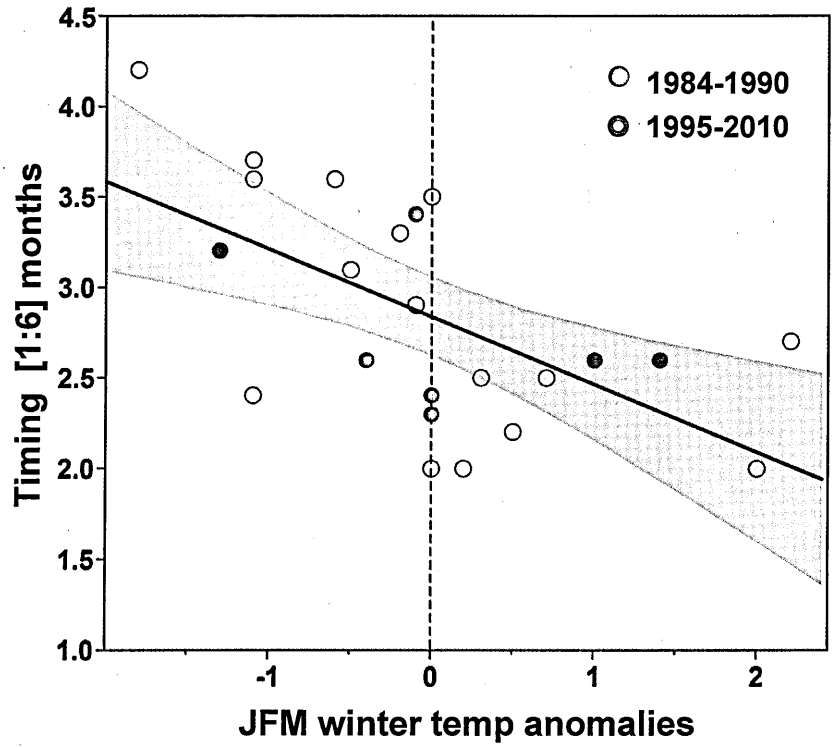

Figure 4.14 Timing of central tendency of cirriped larvae as a function of winter (JFM) temperature anomalies. Shaded region indicates the 95% confidence area for a bivariate fit of phenophase of timing by temperature anomalies.

Among less abundant copepods, the timing of *O. setigera* was negatively correlated with autumn anomalies of temperature (Fig. 4.15), while the timing of *F. rostrata* was positively correlated with spring anomalies (Table 4.3, Fig. 4.16). The taxon *Clausocalanus* spp. males, which includes the males of eight congeneric species, had a timing negatively correlated with surface and depth integrated temperature anomalies (Fig. 4.17).

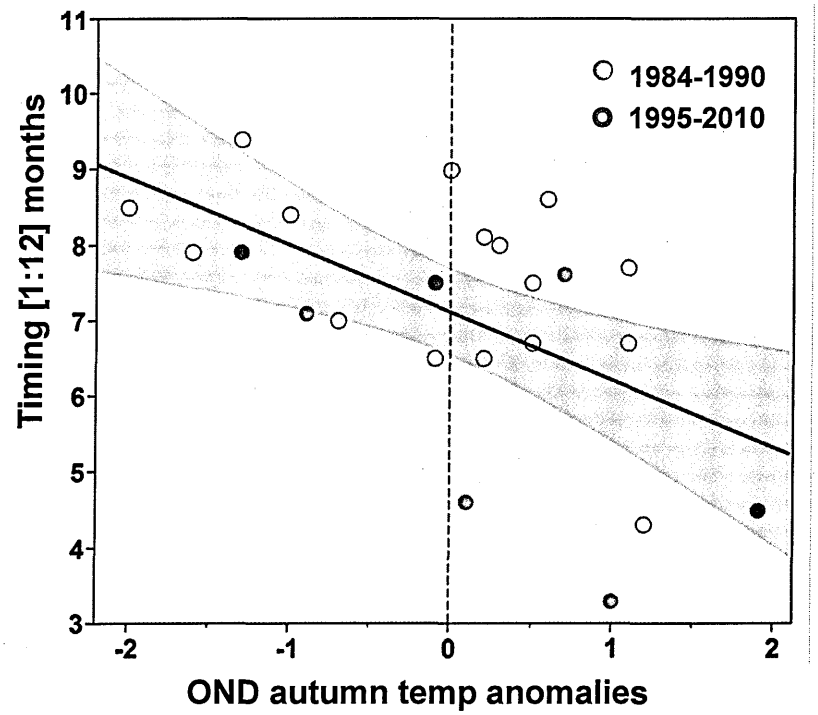

Figure 4.15 Timing of central tendency of *Oithona setigera* (females) as a function of autumn (OND) temperature anomalies. Shaded region indicates the 95% confidence area for a bivariate fit of timing by temperature anomalies.

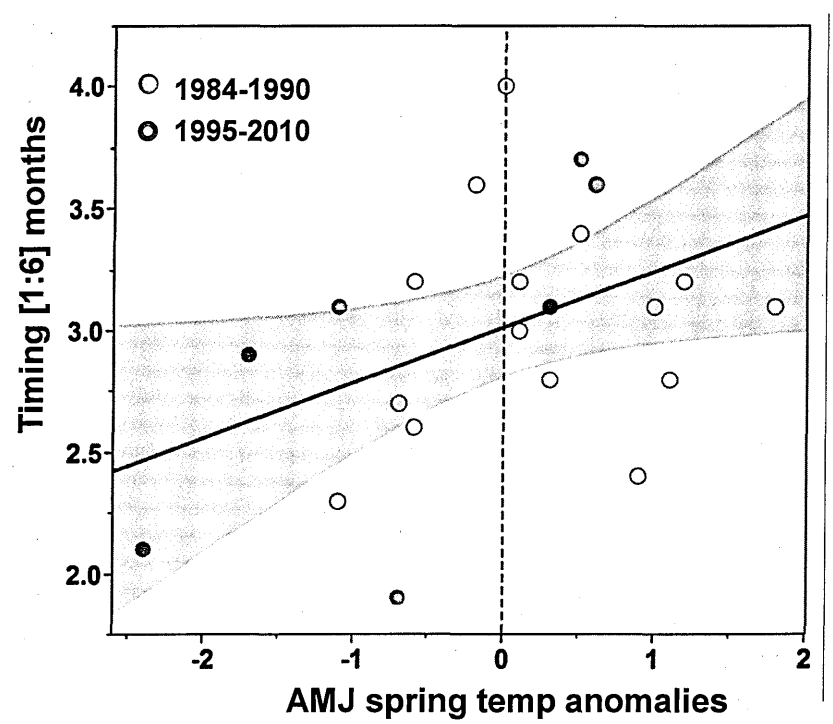

Figure. 4.16 Timing of central tendency of *Farranula rostrata* as a function of spring (AMJ) temperature anomalies. Shaded region indicates the 95% confidence area for a bivariate fit of timing by temperature anomalies.

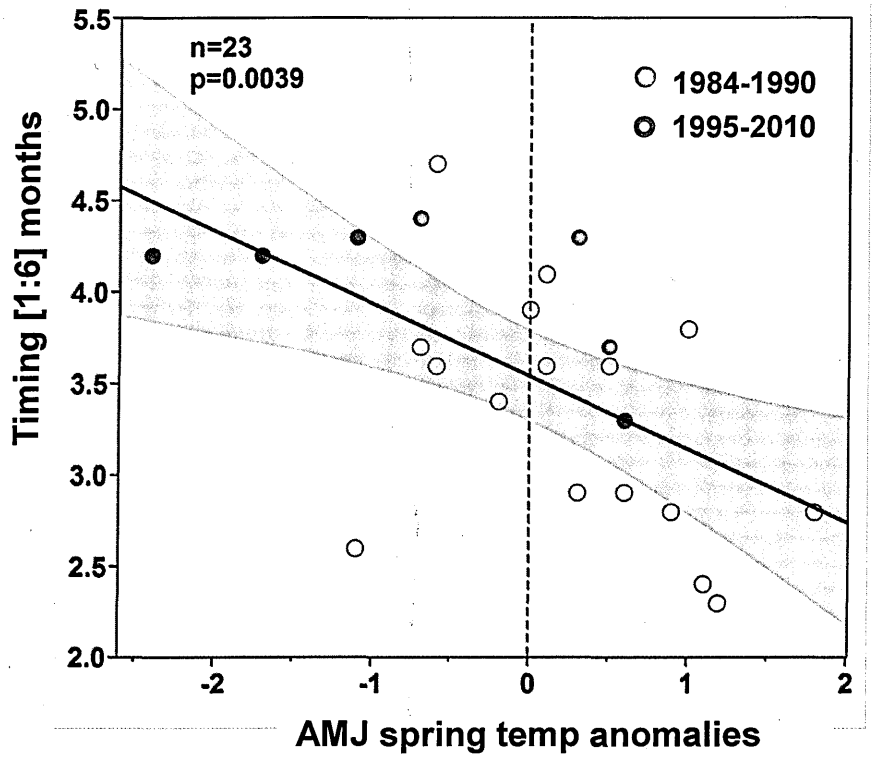

Figure. 4.17 Timing of central tendency of *Clausocalanus* spp. males as a function of spring (AMJ) temperature anomalies. Shaded region indicates the 95% confidence area for a bivariate fit of timing by temperature anomalies.

4.3.2 Zooplankton phenology and chlorophyll *a* anomalies

Significant correlations between phenology of zooplankton taxa and seasonal anomalies of surface and integrated chl *a* are presented in Table 4.4. Figures presented are highlighted in green to delineate the parameter of interest in this case chlorophyll.

Table 4.4 Significant correlations between zooplankton phenological events and seasonal anomalies of surface and integrated chl *a* . Zooplankton categories are listed alphabetically by group and by copepod species. Pairwise correlation coefficients (*r)* and significance at * ($p \leq 0.05$).

category	phenology event	seasonal anomalies	depth	*r*
Pisces larvae	timing [1:6]	spring	integrated	-0.48 *
Salps	timing [1:12]	spring	surface	-0.56 **
Salps	timing [1:12]	spring	integrated	-0.56 **
Salps	start phase	spring	surface	-0.52 *
Salps	start phase	spring	integrated	-0.53 **
Total copepods	timing [1:6]	summer	integrated	0.44 *
Total zooplankton	timing [1:6]	summer	integrated	0.42 *
Clausocalanus arcuicornis females	middle phase	winter	surface	0.66 **
"	middle phase	spring	surface	0.61 **
"	middle phase	winter	integrated	0.63 **
"	middle phase	spring	integrated	0.63 *
Clausocalanus spp.	timing [1:6]	spring	surface	0.42 *
"	timing [1:6]	spring	integrated	0.48 *
Farranula rostrata	timing [1:6]	spring	integrated	-0.44 *
Isias clavipes	timing [1:12]	spring	surface	0.45 *
"	timing [1:12]	spring	integrated	0.45 *
Nannocalanus minor	timing [7:12]	summer	surface	-0.48 *
Oithona longispina	start phase	spring	surface	0.47 *
"	start phase	spring	integrated	0.49 *
Oithona nana	start phase	winter	integrated	0.43 *
Oithona setigera females	timing [1:12]	summer	surface	-0.44 *
"	timing [1:12]	summer	integrated	-0.43 *
"	timing [1:12]	autumn	integrated	-0.57 *

Total zooplankton and total copepods had a positive correlation with summer chl *a* anomalies, i.e., the timing occurred later when anomalies were positive (Fig.4.18). In both cases, the higher chl *a* anomalies in the first part of the time series (particularly in 1986) determined the overall trend towards later timing.

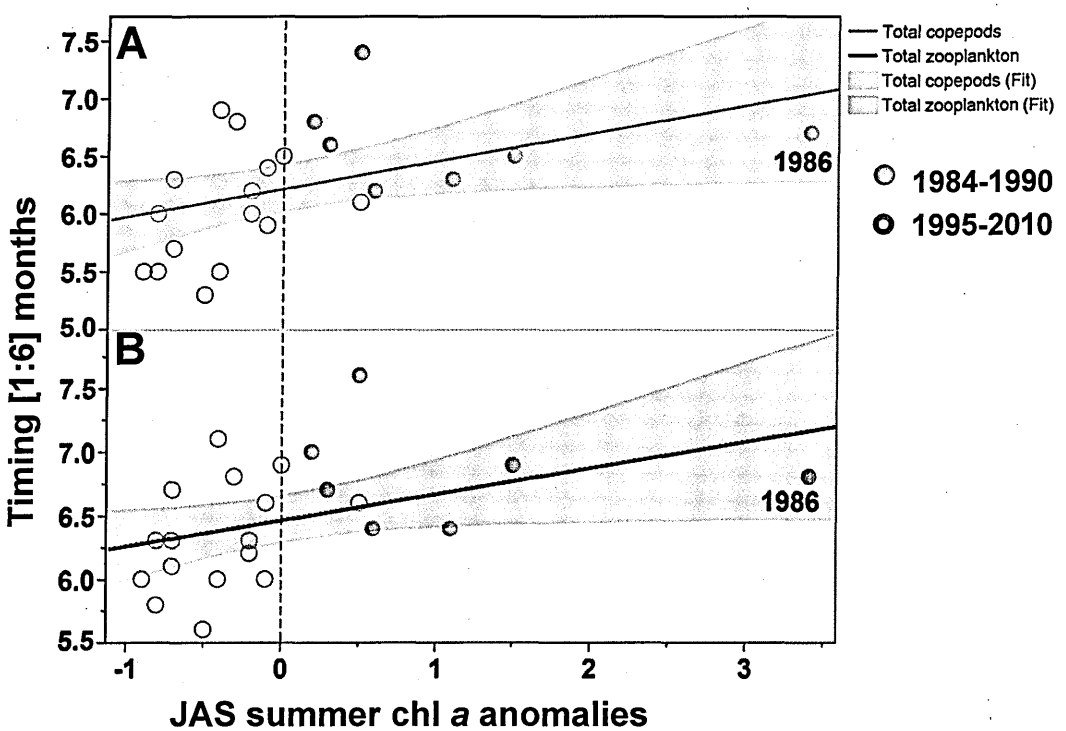

Figure 4.18 Timing of central tendency of (A) total copepods and (B) total zooplankton as a function of summer (JAS) integrated chl *a* anomalies. 1986 is labeled to indicate a particularly high seasonal chl a anomaly. Shaded region indicates the 95% confidence area for a bivariate fit of timing by chl *a* anomalies. Note that overlapping timing occurred in 1997 and 2007 showing only 15 open circles.

Salps had the start phenophase and timing negatively correlated with integrated spring anomalies, i.e. these phenological events occurred earlier with positive anomalies (Fig. 4.19). Pisces larvae and eggs were negatively correlated with spring chl *a* anomalies (Fig. 4.20). The timing occurred between March and mid-April in the first part of the time series, and shifted towards May in the second part (see supporting Table 3.5).

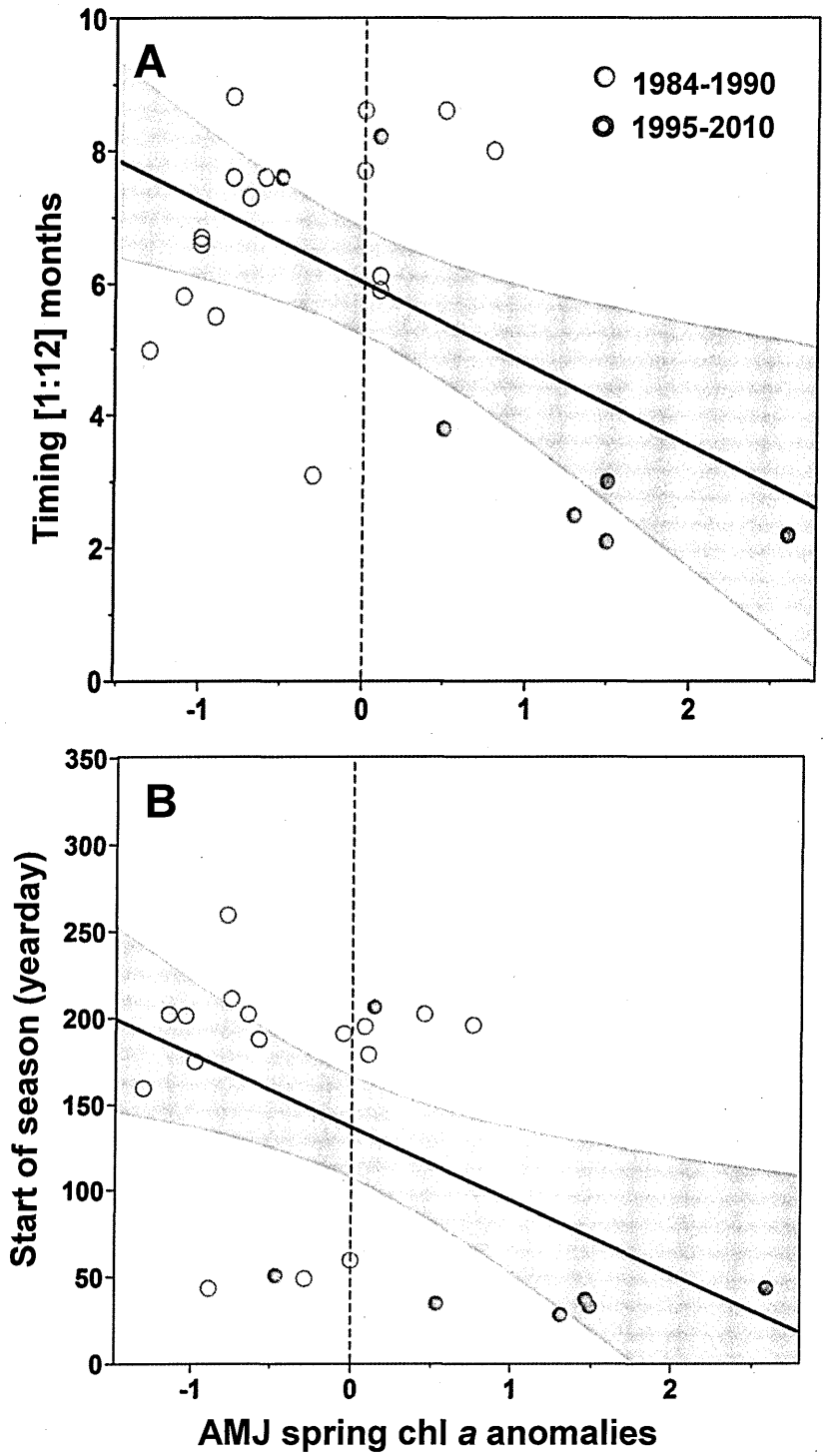

Figure 4.19 (A) Timing of central tendency of salps and (B) start phenophase as a function of integrated spring (AMJ) chl *a* anomalies. Shaded region indicates the 95% confidence area for a bivariate fit of phenological events by chl *a* anomalies.

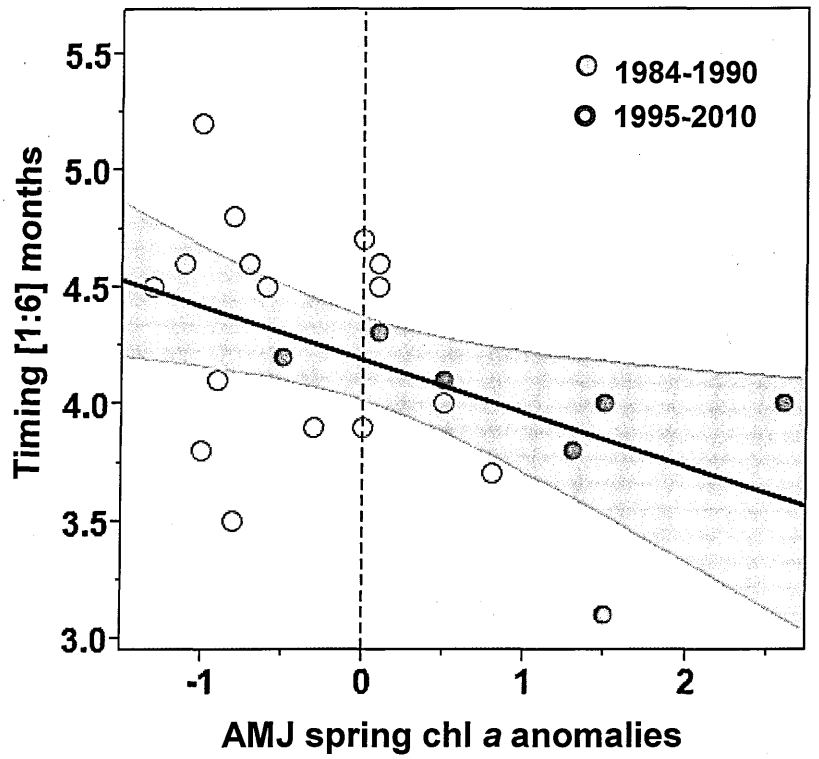

Figure 4.20 Timing of central tendency of Pisces larvae + eggs as a function of spring (AMJ) integrated chl *a* anomalies. Shaded region indicates the 95% confidence area for a bivariate fit of timing by chl *a* anomalies.

Within copepods, 7 species and a bulk category (*Clausocalanus* spp. males) showed significant changes in phenological events that were significantly correlated to seasonal chl *a* anomalies (Table 4.4). The middle phenophase of *C. arcuicornis* females were correlated with winter and spring anomalies; three consecutive years, i.e., 1984, 1985 and 1986, were responsible for this relationship (Fig 4.21A, B). When these years were included in the correlation analysis, the relationship was significantly positive, while when they were removed, the relationship became negative (Table 4.4). With 3 years removed, the relationship remained significantly negative ($p=0.0090$) with spring anomalies. The significance values reported in Table 4.4 include the three years. The middle phenophase of *C. arcuicornis* females was positively correlated with both surface and integrated chl *a* (Table 4.4).

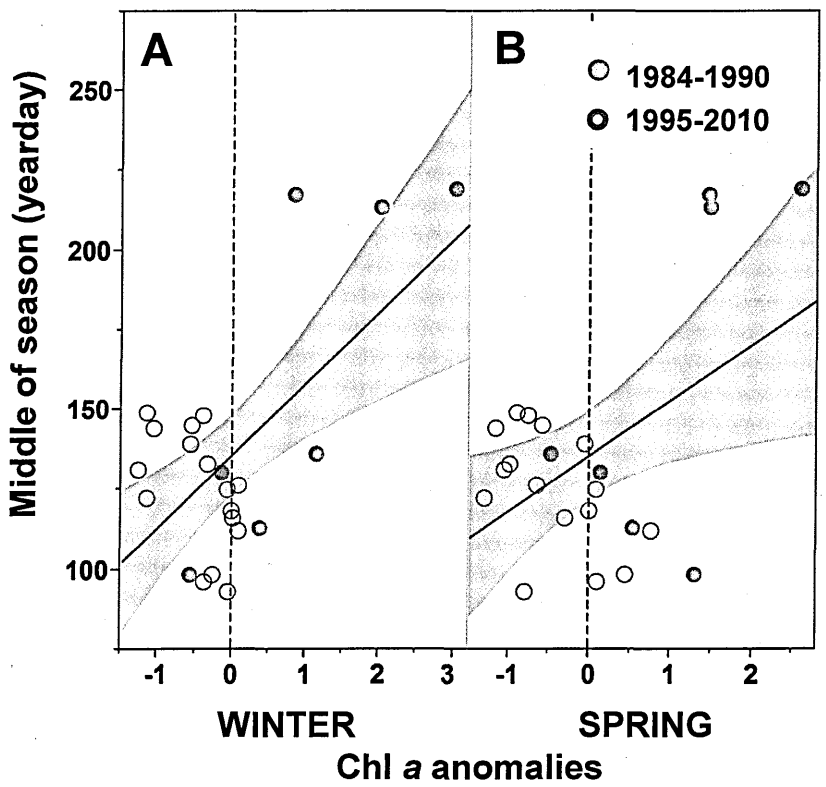

Figure 4.21 Middle phenophase of *Clausocalanus arcuicornis* females as a function of winter (A) and spring (B) chlorophyll anomalies. Shaded region indicates the 95% confidence area for a bivariate fit of phenophases by chl *a* anomalies.

Three species within the genus *Oithona* (*O. longispina, O. nana* and *O. setigera*) had phenological events significantly correlated with chl *a*. The start phenophase of *O. longispina* and *O. nana* was positively correlated with chl *a* anomalies in spring and in winter, respectively (Fig. 4.22A and B). The timing of *O. setigera* was negatively correlated with summer and autumn anomalies (Fig. 4.23).

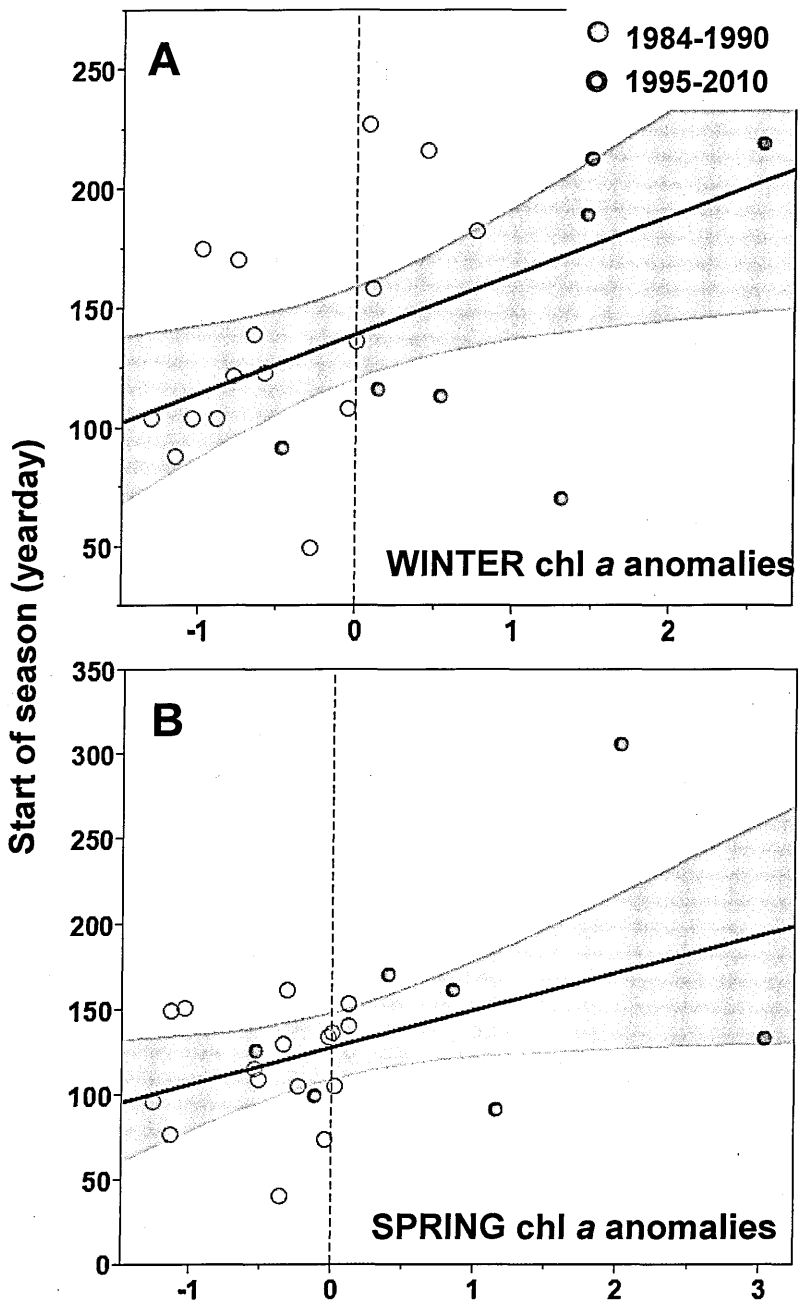

Figure 4.22 Start phenophases of *Oithona longispina* (A) and *Oithona nana* (B) as a function of spring (A) and winter (B) chl *a* anomalies. Shaded region indicates the 95% confidence area for a bivariate fit of start phenophases by chl *a* anomalies.

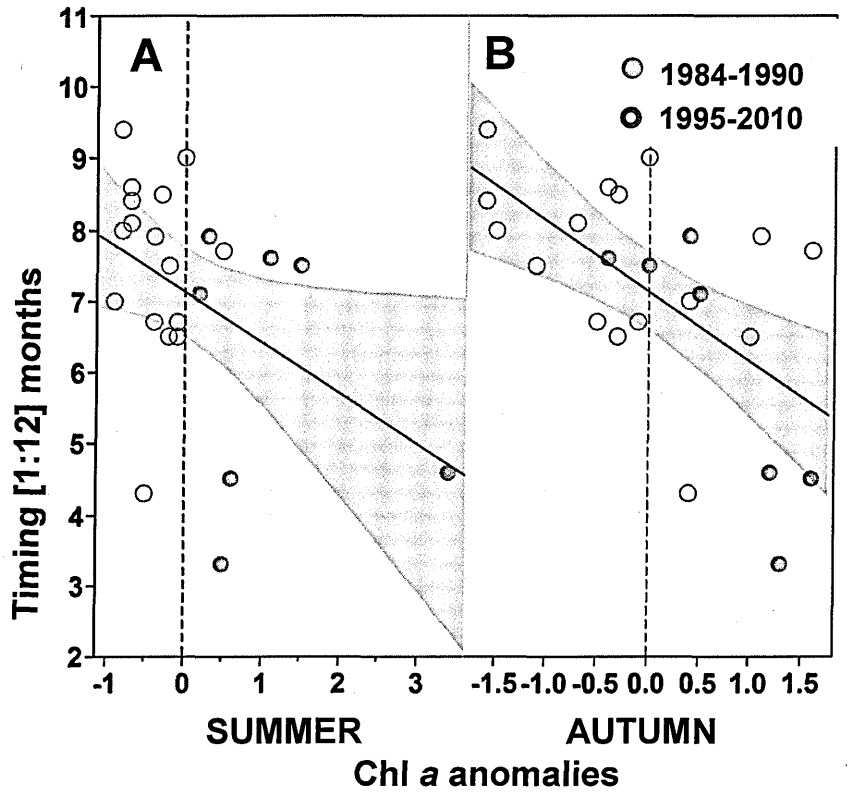

Figure 4.23 Timing of central tendency of *Oithona setigera* females as a function of summer (A) and autumn (B) chl *a* anomalies. Shaded region indicates the 95% confidence area for a bivariate fit of timing by chl *a* anomalies.

4.4 Chapter summary

In summary, the following relationships emerged in the correlation of long-term plankton phenology and environmental parameters.

- Out of the 7 species showing a change of the onset of phenophases (start 25% of cumulative abundance), 3 *C. tenuissimus, H. niei* and *D. faculiferum* showed a correlation with salinity anomalies. *C. tenuissimus* and *D. faculiferum* occurred earlier when salinity anomalies were positive, and *H. niei* occurred later when salinity anomalies were positive.
- Of the 9 phytoplankton taxa showing changes in duration of occurrence, 5 diatom taxa showed coherent longer duration which had significant positive correlations with positive temperature anomalies.
- Few zooplankton categories showed relationships with temperature however, total copepods, cirriped larvae, and copepod taxa *Oithona setigera,* and the genus *Clausocalanus* spp. showed a negative correlations between the timing of central tendency in the first half of the year and positive temperature anomalies.
- Changes in timing of total copepods and total zooplankton timing were positively correlated with chl *a* anomalies in summer.

Chapter 5

DISCUSSION

5.1 Long-term changes in plankton phenology

The first synoptic view of long-term plankton phenology at stn MC revealed that relatively few taxa have significantly changed their phenology over a 23 year time period (Appendix Table 5.1). However, among the phytoplankton and zooplankton taxa that showed changes in different aspects of their phenology, most had coherent patterns of change in the month of peak abundance and the timing of central tendency which supports the premise put forth that species-level patterns of abundance and phenology can be used to understand community-level patterns of change (Durant *et al.*, 2005, Ji *et al.*, 2010).

Despite seasonal and inter-annual variability in phytoplankton and zooplankton populations, the general composition of plankton assemblages did not change considerably from previous synthesis carried out by others (see Chapter 1, §1.3), suggesting that the plankton community structure in the Gulf of Naples has not undergone a regime shift observed in other plankton ecosystems such as in the Adriatic Sea (Conversi *et al.*, 2009).

The seasonal succession patterns that emerge from these results depict a rapid transition in the phytoplankton community from diatoms to dinoflagellates in the spring, followed by flagellates and coccolithophores in the second half of the year. These seasonal successional patterns followed a well described paradigm in coastal phytoplankton communities whereby their succession is governed by the physico-chemical environment in which small chained and colonial forming diatom species generally favor well-mixed, turbulent, nutrient replete conditions typical of winter months, and transitional periods from late autumn and winter to spring months, whereas smaller sized flagellates favor stratified conditions in summer and intermittent periods of stability (Cushing, 1989, Margalef, 1978, Reynolds, 2006).

Typical zooplankton succession begins with a late winter peak of appendicularians in March, followed by copepods in April, then a peak of cladocerans and a secondary peak

of copepods in August. The relative contribution of dominant mesozooplankton taxa and groups did not change significantly from analysis carried out on the time series until 2006.

5.1.1 Changes in seasonal peak occurrence

Although many dominant taxa occurred regularly throughout the time series, numerous taxa showed change in the timing and magnitude of seasonal peak abundance. More than 20% of the 192 plankton taxa showed change in the month of seasonal peak abundance (Table 3.3). Zooplankton showed 3% higher change in the peak month than phytoplankton.

Diatoms, other flagellates and dinoflagellates maintained the month of peak abundance. Total diatoms acquired a secondary peak in August in the second part of the time series, while total coccolithophores shifted from a bimodal peak cycle to a single peak of abundance in October showing an overall later shift in the seasonal phenology. This change was marked by a single dominant coccolithphore species – *Emiliania huxleyi* which also presented the same pattern in various phenological events. This unusual shift in phenology illustrates how species-level patterns can shape the change observed in functional groups.

Several numerically abundant diatoms (*Skeletonema pseudocostatum*, *Minidiscus comicus*, *Thalassiosira* cf. *allenii*, the *Pseudo-nitzschia delicatissima* complex, and *Chateoceros "curvi-curvi"*) all had an earlier pattern in the month of peak abundance in the second period of the time series. In contrast, several species shifted to a later pattern of peak abundance; *C. tenuissimus*, *C. throndsenii*, *Pseudo-nitzschia pseudodelicatissima*, and *Ceratulina pelagica*. Changes in diatoms were observed in spring populations whereas in the case of other flagellates and coccolithophores, changes included taxa in spring and autumn.

Total zooplankton did not show a change in long-term seasonal peak month whereas total copepods were earlier in accordance with changes in individual copepod

species. The genus category of *Calocalanus* spp. followed by the genus level *Oithona* spp. category and numerous species within the genus *Clausocalanus* showed change in the month of seasonal peak.

In addition to changes in the month of seasonal peak, some taxa also presented a long term change in abundance. Total diatoms had a significant negative trend in the first period which did not persist over the long-term. However, diatoms and other flagellates increased in abundance in the second period. Total zooplankton and appendicularians significantly increased abundance in the second part of the time series. Diatoms - *Skeletonema menzelii*, *Pseudo-nitzschia galaxiae*, *Chaetoceros simplex, Pseudo-nitzschia multistriata* and *Leptocylindrus mediterraneus* all increased in abundance. Only *C.curvisetus* and *T.mediterranea* decreased in abundance. Although these are relatively few taxa overall, they represent 6.25% of the average annual diatom community at stn. MC.

In other cases, new taxa appeared while some disappeared. The diatom species *Skeletonema menzelii* did not occur with regular frequency until the second part of the time series. Abundance of *S. menzelii* was recorded in 1987 and 1998 and a substantial peak in 2009 and 2010. Additionally, the diatom *Pseudo-nitzschia multistriata* became a regular part of the taxonomic record after 1995. The copepod *Acartia margalefi* was part of the regular taxonomic record in the first part of the time series, but was not present in the second part while *Acartia negligens* was not recorded regularly until 1999. The disappearance and subsequence appearance of these select taxa was not an artifact of under-sampling since the sampling frequency after 1995 increased from fort-nightly to weekly, thus providing greater temporal resolution with which to detect changes in abundance and phenology.

5.1.2 Common patterns of phenology

Following an approach implemented by (Edwards & Richardson, 2004) to determine whether long-term change in phenology resulted in a trophic mismatch, the results in Chapter 3.5 present the first integrated view of change in combined plankton phenology at stn MC. The overall pattern revealed that copepods have become earlier within the zooplankton community, while phytoplankton taxa did not show a coherent pattern of earlier or later timing. Remarkably absent from the community were changes in the timing of summer plankton taxa. The negative trend observed in the combined trophic levels suggests that these select taxa have advanced their long-term phenological timing.

These results were similar to the early which showed a coherent early pattern in spring and autumn taxa with variability among the individual groups (Edwards & Richardson, 2004). In contrast, this first depiction of long-term change in the plankton covered more trophic levels including coccolithophores and flagellates which are an important component of the phytoplankton community at stn MC. Considering that few phenological studies have utilized this approach on a wide range of taxa, these results reflect a wider view of the complete plankton community, however, the interpretation of change relative to a single year may not accurately capture the general patterns of change.

Since inter-annual variability in population abundance and phenology is a consistent feature of plankton populations, this representation of long-term change in phenology relative to the beginning of the time series (1984) was highly influenced by the first year. Whereas there was no relationship observed among the 12 taxa in the first part of the time series relative to the start year, the second part of the time series revealed an earlier pattern relative to 1995. Therefore, the choice of a reference dependent variable depends on the degree of inter-annual variability and reliability of phenology data. The soundness of phenological data will be considered in 5.2.

5.1.3 Plankton phenology related to seasonal water column dynamics

Phytoplankton taxa showed significant relationships primarily with seasonal and monthly salinity anomalies while zooplankton showed strong relationships with chlorophyll *a* anomalies (Chapter 4). Although temperature is a well documented physical parameter known to impact physiological processes and accelerate growth rates, (Aberle *et al.*, 2012, Genner *et al.*, 2009, Mackas *et al.*, 2007) it did not appear that phenological change resulted from a direct association with temperature. It is likely that seasonally driven changes in temperature and salinity acting in concert are the dominant mechanisms behind water column structure and resulting physico-chemical conditions favorable for different structured plankton communities. The relationship between hydro-climatic forcing and phenology is likely indirect (Caron & Hutchins, 2013).

At the seasonal scale, changes in phytoplankton phenology were correlated with spring and summer salinity anomalies with the exception of a less abundant diatom species (*C. affinis)* which was correlated with winter salinity anomalies. The phenology of several populations occurred later when salinity anomalies were higher than average. There was no apparent pattern among the populations that were correlated with salinity however both *Minidiscus comicus* and *Heterocapsa niei* had distinct patterns of phenology. The relationships between phenology and seasonal anomalies were present within the monthly anomalies that marked the specific window for phenology. In the case of *C. tenuissimus* which presented changes in the onset of phenology and in the timing, a seasonal relationship with spring (AMJ) salinity was shaped by a correlation in March and April and with May temperatures. Whereas this bimodal taxa peaks in May and August, *C. tenuissimus* showed strong relationships with environmental parameters only in spring. The recurrent timing of *C. tenuissimus* in August, and the strong correlation with summer temperature anomalies suggests that despite the strong correlation, the timing did not reflect a change but rather a stable pattern of phenology irrespective of the warmer surface water temperatures in summer.

It is worthy of consideration whether or not the populations that were strongly correlated with salinity are indicative of off-shore populations or different seasonal water masses. The numerous relationships between phytoplankton and spring conditions suggest that phenology could be driven by larger physico-chemical conditions. During winter, hydrographic conditions in the Gulf of Naples result in a well-mixed water column and frequent flushing of the coastal region with deeper off-shore waters more typical of oligotrophic systems. In this case, we would expect that nutrients are lower and salinity is higher, however the accepted paradigm at stn MC is that nutrients are always replete and are not the primary mechanism driving growth and accumulation of phytoplankton populations.

The consequences of strong seasonally driven community structure reflect a scenario with two different systems modulated by both biological community composition and physico-chemical conditions. The first system is a shorter traditional food chain supported by microalgae >5 μm and copepods which represents a direct link to higher trophic levels such as fish. The second system is a microbial mediated food chain dominated by heterotrophic microflagellates and small picophytoplankton that are more typical of stratified, oligotrophic systems.

5.1.4 Zooplankton phenology related to seasonal chlorophyll

Among the numerous relationships between zooplankton phenological events and environmental anomalies, very few showed coherent signatures of change in relation to temperature or chl *a*. However, some taxa and groups are worthy of highlighting for their known importance or ecological role within the zooplankton community. Salps showed a strong signature of phenological change with later trends in all phenophases and a longer duration, and also showed significant relationships with temperature in spring and chl *a* in summer. The different seasons in which these relationships occurred suggest that both aspects could be linked to the later onset in summer months and may be a possible driver

of phenology. The genus level *Clausocalanus* spp. males also showed strong relationships with temperature and chl *a* but few only showed a change in earlier timing in the spring. Although the phenology of total copepods as a bulk group did not reveal change in more than 1 phenological event, there were strong relationships between the timing of central tendency and temperature and chl *a* that showed a coherent response to summer conditions when copepods have a peak abundance.

Phytoplankton and zooplankton cycles of abundance represent two different trophic levels. Phytoplankton as primary producers have short life cycles and exhibit rapid responses to surrounding conditions such as light, temperature and nutrient availability, whereas zooplankton as grazers on phytoplankton and general consumers constitute a higher trophic level with a nearly constant supply of varied food opportunities.

5.2 Assessment of plankton phenology at stn MC

A thorough assessment of these results is limited to the scope of understanding both plankton ecology and phenology and may be more appropriate for a comparative analysis of phenology with another plankton time series, but nevertheless we should consider the relevant changes that were identified and the significance when making ecological interpretations, and finally we should accept the inherent limitations in determining plankton phenology. Unlike terrestrial systems, planktonic ecosystems experience rapid turnover times in both biological production and chemical cycling. Therefore, the time scales for phenological events and long-term change must be adjusted accordingly. Phytoplankton (nano and microphytoplankton) typically double their growth rate between 8-15 intervals which makes the window of weekly sampling time in a time series adequate for capturing a recurrent population. Zooplankton exhibit a wide range of life history traits and generally have longer cycles depending on the functional group, and therefore their populations also persist over weekly time intervals.

5.2.1 Limitations in identifying plankton phenology

Methodological sampling and statistical analysis are two obvious sources of potential bias and error in the identification of plankton phenology. The use of linear models to assess non-linear systems has been criticized by (Hudson, 2010), but yet it is still widely applied in large scale studies of plankton phenology in relation to climate (Mackas & Beaugrand, 2010). Although these results have been tested for reproducibility and statistically verified, the synthetic comparison of change in multiple phenological events revealed some obvious inconsistencies in the analysis (Appendix Table 1).

Sampling methodologies for plankton have been regularly maintained at stn MC. This results in a reliable window of time (1 week intervals) with which to capture the phenology of most populations with the exception of sporadic, rare or bloom taxa that do not persist. The plankton communities are sampled at surface for phytoplankton, and using

a vertically integrated water column tow for zooplankton. Hence, the general patterns of each dataset reveal sporadic, spiky patterns of phytoplankton abundance and phenology and a bias towards better sampling resolution during summer when the dominant phytoplankton populations occur in stratified surface waters, where as the general zooplankton community is sampled on a vertical scale and thus the sample captures a more comprehensive snapshot of the community throughout the annual cycle. Phytoplankton sampling methods lack vertical resolution, thus abundance measurements reflect only surface measurements and as a result may also underestimate biomass and secondary zooplankton grazing rates (Sutor & Dagg, 2008).

As a result of a 4 year gap in sampling, most statistical analyses required analysis in two different parts of the time series spanning 7 and 16 years respectively. The results presented in this work imply that some changes occurred over the long-term; however caution should be exercised in the statistical interpretation of these changes. As a result of a more than 2-fold difference in the number of degrees of freedom when comparing two parts of the time series, it is likely that trends were un-detected in shorter periods of time, and that decadal scale trends are more suitable.

Non-coherent patterns of phenological change (reported in results Appendix A1) must be scrutinized on a species-group basis in order to further refine the understanding of the observed change and possible drivers. The integration of these results into a comprehensive view of the community failed to present another important aspect of phenology – *that which has not changed.* This non-change is less often considered with respect to ecological questions driven by climate change. Instead, a large portion of the plankton community at stn MC remained unchanged in the long-term. A possible limitation of these results may be the definition of ecological change and the relevant time scales with which we measure change.

5.3 Comparative results with other time series

5.3.1. Comparison with Helgoland time series

Despite limitations in carrying out long-term studies of phenology, there are several existing time series in both marine and freshwater aquatic systems that have addressed plankton phenology using long-term taxonomic plankton and environmental datasets. The Helgoland time series running from 1962 is a rich marine data set that covers both marine biota and hydography from the German Bight and has a temporal resolution of 51 years. The dataset has undergone quality control checks to maintain inter-annual comparability. Additionally, a buoy monitoring program was introduced in 2002 to monitor the introduction and settlement of potential invasive species. From a recent publication (Wiltshire *et al.*, 2010), the long term salinity and annual mean tempereratures increased (the latter by an average of 1.67°C) carried out in an earlier publication by (Wiltshire & Manly, 2004). This increase in temperature caused a shift in diatom densitities and an increase in the number of large diatom taxa. Within the zooplankton community, the appearance of the nuisance ctenophore *Mnemiopsis leidyi* signaled a change in both population abundance and diversity in the plankton community. In addition to these changes, multiple regression analysis confirmed that only winter temperatures had a significant effect on the the MDD (Mean Diatom Day, which is used to identify the timing of the overall bloom in different periods), and FEBD (First Exponential Bloom Day). Temperature appears to be the most important environmental parameter directly influencing copepod phenology; acting as a significant driver on changes linked with increasing temperatures (Wiltshire *et al.*, 2008).

Although the most important factor governing both larval dispersion and biological communities at Helgoland is the hydrography which controls water transport patterns in the German Bight, larger climatic patterns also appear to play an important role such as evidenced by a regime shift change that was identified in the late 1980's and influenced some of the dynamics seen in analysis carried out before 2010 (Wiltshire & Manly, 2004,

Wiltshire *et al.*, 2008). Whereas earlier (until 2004) the time series records revealed a clear warming trend and a regime shift pattern shows a characteristic shift in the biotic or abiotic conditions (Steele, 1998).

Despite the variability in phenology of both phytoplankton and mesozooplankton spring phytoplankton bloom dynamics did not change when the long-term average is considered (Wiltshire *et al.*, 2008). In comparison to the changes observed in phenology at stn MC, many spring diatom taxa were delayed in concert with changes observed in the spring phytoplankton community, there was not an expected earlier when warmer pattern. Therefore, the overall larger community of diatoms showed an earlier trend, but the individual numerically dominant species such as *Chaetoceros socialis* and *C.tenuissimus* were later in their phenology.

This earlier work on the Helgoland time series concluded that the plankton community was resilient because the timing of the spring phytoplankton bloom had remained the same. In concert with a synthesis of mesozooplankton community structure carried out until 2006 (Mazzocchi *et al.*, 2012), the LTER-MC time series was not tested for a change or regime shift in the same period likely due to the presence of a gap in sampling in or around the same time period in which the Mediterranean time series in Trieste registered a shift due to climate level shifts in temperature the Northern Hemisphere (NHT) around 1986-1987 (Conversi *et al.*, 2010, Conversi *et al.*, 2009).

5.3.2. Comparison with Trieste- Adriatic Sea time series

Similar to the Gulf of Naples time series but over a longer time span, the Gulf of Trieste time series in the Adriatic Sea was sampled over a 36 year period from 1970 to 2005 with a 5-year gap between 1981 and 1986 constituting a time series with two distinct periods that showed significant changes in timing and abundance of mesozooplankton copepod species (Conversi *et al.*, 2009). In many cases, copepod taxa showed a forward shift in maximum peak timing in contrast to the phenology of Gulf of Naples taxa which

was not exclusively earlier or later (see Table 3.4, 3.5, 3.6 or Appendix A1 results). However, in their 2009 study the comparative results from the timing of central tendency (Table 3.6) showed that the predominant trend in the Naples time series was towards an earlier phenology whereas the bimodal genus *Clausocalanus* spp. showed a shift in earlier winter/spring timing nearly towards half of a month (-0.4) and the summer fall timing was more than a month and a half later (+1.6) (Table 2 in Conversi *et al.,* 2009). In nearly all 7 unimodal taxa (group, genera and species level) from the Trieste time series, the timing of peak of maximum abundance occurred later as much as up to 4 months in the case of *Corycaeus* spp. and with the exception of *Centropages typicus*, and *Temora longicornis* which were more than 1 month earlier (-1, and -1.6) respectively.

Although the phenological results were mainly focused on the dominant copepod taxa, there were sparse comparisons to be made between the species commonly associated with the Adriatic Sea and those which also exhibited changes in phenology in the Gulf of Naples. There were some relevant changes in long-term abundance between both time series which in some cases were significantly positive in the second period of the time series in both cases: eg. *Euterpina acutifrons* (+ positively increased), *Paracalanus parvus* significantly increased in abundance in the Trieste time series but not over the 16 year time span tested for the Gulf of Naples. Numerous other taxa including total copepods also increased in abundance, while only total zooplankton (mainly driven by increases in appendicularians, gastropods, and chaetognaths) increased in the Naples time series. In general, after changes in abundance and phenology were recorded after 1987.

In this earlier approach, the authors related changes observed in the abundance and phenology of the zooplankton community with general warming of sea surface temperature and other circulation patterns that were indicative of a change in the EMT or Eastern Mediterranean Transient circulation pattern at the basin scale. The SST data was obtained from a meterological station at 2m depth and sampled at daily resolution. The average

seasonal SST was calculated over three month calendar seasons similar to the approach carried out with seasonal temperature anomalies using the Gulf of Naples time series. A 1°C increase in summer and fall SST was associated with a change in the summer peak ofcolder-water taxa such as *Pseudocalanus elongatus* which declined in abundance in the second period of the time series (1988-2005) and a phenological trend towards later timing as evidenced in both late summer timing which shifted from July to mid October between the 1970 and 2005 and between April and March in the first half of the year.

A similar phenomenon occurred in the Gulf of Trieste as with the Gulf of Naples where some rare taxa appeared and others completely disappeared over 30 years of time series records (Garcia-Comas Rubio, 2009). Changes in diversity and community level composition are believed to reflect the influence of environmental change as evidenced in the relationship with temperature in previous studies. Additionally, an increase in salinity between 1965 to 1985 was related with an increase in primary production. The entrance of higher salinity Mediterranean Waters into the Gulf of Trieste was related to a decadal oscillation and correlated with the NAO (North Atlantic Oscillation) climate pattern (Garcia-Comas Rubio, 2009). The changes in temperature and salinity related to both local and global regional European climatic patterns confirm that the analysis carried out on the phenological relationships between plankton and temperature and salinity of Gulf of Naples plankton present a robust set of data analysis that can be compared taxa from other phenological studies in the Mediterranean.

5.3.3. Comparison with L4 time series

Although not part of the regularly sampled Mediterranean time series, insights into plankton phenology from the L4 time series located in the Western English Channel were highlighted in a special issue of JPR (*Journal of Plankton Research*). Over a 15 year period from 1992 to 2007, weekly water samples from the Western English Channel were collected as part of the L4 time series station sampling (Widdicombe *et al.*, 2010). I have

chosen to include L4 in this comparative section because the station is a characteristic temperate, coastal station which has similar hydrographic features (to the Gulf of Naples) with well mixed waters in autumn and winter months (with SST around 8°C) and abundant nutrients similar to stn MC. In spring and summer, weak water column stratification begins in combination with decreasing nutrients and increasing SST with a typical peak of 18°C. In a long-term study carried out as a PhD thesis and later incorporated into the special issue on plankton dynamics, phenological changes were investigated for some of the dominant zooplankton taxa (Eloire, 2010, Eloire *et al.*, 2010). Using comparable methods as Greve (outlined in Methods Chapter 2). Although detailed analysis was only presented for two dominant taxa: *Acartia clausi* and *Temora longicornis*, the analysis revealed an earlier appearance (anticipated phenology), and a reduced duration characterized by the fact that their populations declined or disappeared two months earlier. *Temora longicornis* although not present in the Gulf of Naples time series is a taxon recorded in the Adriatic time series and showed a similar earlier trend of nearly 1 month in anticipation confirming that spatial separation and regional climatic differences may not fully explain the observed differences or similarities in phenology. The abundant taxa *Acartia clausi* is recorded at stn MC with a clear dominant peak in summer in agreement with the seasonality at L4. The most significant change in phenology at L4 was the presence of diatoms that became more abundant in summer instead of autumn. A striking change in seasonality of total diatoms was also observed in the Gulf of Naples where taxa that were once abundance in the late spring months of April through June with a dominant peak in May acquired a secondary late summer peak in August (pg. 63 Fig. 3.2). A rise of 0.6°C per decade in the western English Channel was identified as the primary driver of the observed phenological changes of the copepod species. Although nutrients were not part of the analysis carried out on the Gulf of Naples the increase in summer and early autumn temperature and significant trend

in both spring and summer temperature anomalies (see Table 4.1A and B) has implications for the breakdown and general structure of the water column and nutrient distribution.

5.4 Proposed future work: towards an understanding of plankton phenology

From this first view of plankton phenology, the primary patterns of phenology have been identified using numerical and statistical methods that can be cross-applied to other planktonic time series in a similar manner. Despite a growing tool-box of sampling and data analysis methods, there are some considerations for future studies. In practical terms, predictive modeling efforts that incorporate climatic indices should also incorporate aspects of plankton life history and phenology in order to resolve seasonal dynamics and phenological responses to changing climate conditions.

In addition to including phenology of functional groups and individual species into climate models, a regional assessment index should be developed for time series or monitoring programs in which key taxa (known to exhibit a phenological response in the long-term) are followed closely and their phenological patterns are updated. In a coastal time series, a taxonomic database of plankton abundance with corresponding dates of occurrence might incorporate a monthly assessment to determine whether the phenology of taxa was later, earlier, or remained unchanged. Following approaches being developed for citizen science efforts in terrestrial systems, existing platforms can be utilized to maintain a long-term record of phenology.

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APPENDIX

A.1 LIST OF PHENOLOGICAL CHANGES IN PHYTOPLANKTON AND ZOOPLANKTON TAXA AND RELATION WITH ENVIRONMENTAL DRIVERS. Note that this appendix table is available as a sortable data table file available upon permission of thesis author. Changes in phenological timing are represented in (units) and in (+ or -) delayed or anticipated respectively within cells. Relationship with seasonal or monthly environmental anomalies are also represented as (+ or -) delayed or anticipated respectively within cells.

Phenophases (phytoplankton)

community	functional group	category	change in start 25% (days)	change in start 25% (time period)	change in middle 50% (days)	change in middle 50% (time period)	change in end 75% (days)	change in end 75% (time period)
phyto	coccolithophores	*Emiliana huxleyi*						
phyto	coccolithophores	total coccolithophores						
phyto	coccolithophores	undetermined coccolithophores						
phyto	diatoms	*Chaetoceros affinis*						
phyto	diatoms	*Chaetoceros anastomosans*						
phyto	diatoms	*Chaetoceros simplex*						
phyto	diatoms	*Chaetoceros socialis*	+166	II	+191	II		
phyto	diatoms	*Chaetoceros tenuissimus*	+113	II				
phyto	diatoms	*Dactyliosolen phuketensis*						
phyto	diatoms	*Minidiscus comicus*	-243	II	-229	II	-215	II
phyto	diatoms	pennate diatoms >10 µm						
phyto	diatoms	*Proboscia alata*	-198	I				
phyto	diatoms	*Pseudo-nitzschia multistriata*						
phyto	diatoms	*Skeletonema menzelii*	+28	II				
phyto	diatoms	*Thalassionema nitzschioides*						
phyto	diatoms	*Thalassiosira mediterranea*						
phyto	diatoms	*Thalassiosira* spp.						
phyto	diatoms	total diatoms						
phyto	dinoflagellates	*Dinobryon faculiferum*	+99	II				
phyto	dinoflagellates	*Heterocapsa niei*	-104	II				
phyto	dinoflagellates	Naked dinoflagellates >15 µm						
phyto	dinoflagellates	total dinoflagellates						
phyto	flagellates	*Diplostauron cf.elegans*						
phyto	flagellates	*Pachysphaera* spp.			+65	II		
phyto	flagellates	undetermined phytoflagellates >10 µm						

Duration and timing of central tendency (phytoplankton)

functional group	category	change in duration (days)	change in duration (time period)	change in timing (month)	change in timing (time period)
coccolithophores	*Emiliana huxleyi*			+1.2	I
coccolithophores	total coccolithophores			+1.0	I
coccolithophores	undetermined coccolithophores	-49	I		
diatoms	*Chaetoceros affinis*	+106	I		
diatoms	*Chaetoceros anastomosans*	+105	(1985-1990)		
diatoms	*Chaetoceros simplex*	+42	II		
diatoms	*Chaetoceros socialis*			+5.28	II
diatoms	*Chaetoceros tenuissimus*			-0.8	II
diatoms	*Dactyliosolen phuketensis*	-95	II		
diatoms	*Minidiscus comicus*			-7.25	II
diatoms	pennate diatoms >10 µm	+129	II		
diatoms	*Proboscia alata*				
diatoms	*Pseudo-nitzschia multistriata*			-2.75	(1996-2010)
diatoms	*Skeletonema menzelii*			-2.98	II
diatoms	*Thalassionema nitzschioides*			+4.13	I
diatoms	*Thalassiosira mediterranea*	+138	I	-6.53	II
diatoms	*Thalassiosira* spp.	+169	I	-1.9	I
diatoms	total diatoms			-0.4	II
dinoflagellates	*Dinobryon faculiferum*				
dinoflagellates	*Heterocapsa niei*			-1.58	II
dinoflagellates	Naked dinoflagellates >15 µm			-2.1	I
dinoflagellates	total dinoflagellates	-80	I	-1.2	I
flagellates	*Diplostauron cf.elegans*			-1.0	(1999-2010)
flagellates	*Pachysphaera* spp.				
flagellates	undetermined phytoflagellates >10 µm			+2.0	II

Relationship with temperature anomalies (phytoplankton)

functional group	category	relation temp (+,-)	phenology index	month anomaly (temp)	relation temp anom (+,-)	season anomaly (temp)
coccolithophores	*Emiliana huxleyi*					
coccolithophores	total coccolithophores					
coccolithophores	undetermined coccolithophores					
diatoms	*Chaetoceros affinis*	+	duration	April		
diatoms	*Chaetoceros anastomosans*	+	duration	August		
diatoms	*Chaetoceros simplex*					
diatoms	*Chaetoceros socialis*					
diatoms	*Chaetoceros tenuissimus*	+	timing [1:6]	May	-	summer
diatoms	*Dactyliosolen phuketensis*					
diatoms	*Minidiscus comicus*					
diatoms	pennate diatoms >10 µm	+	duration	August		
diatoms	*Proboscia alata*					
diatoms	*Pseudo-nitzschia multistriata*					
diatoms	*Skeletonema menzelii*					
diatoms	*Thalassionema nitzschioides*					
diatoms	*Thalassiosira mediterranea*		timing [1:12]		-	spring
diatoms	*Thalassiosira* spp.	+, -	duration	March, June		
diatoms	total diatoms					
dinoflagellates	*Dinobryon faculiferum*					
dinoflagellates	*Heterocapsa niei*	+	timing [1:12]	May		
dinoflagellates	Naked dinoflagellates >15 µm					
dinoflagellates	total dinoflagellates					
flagellates	*Diplostauron cf.elegans*					
flagellates	*Pachysphaera* spp.					
flagellates	undetermined phytoflagellates >10 µm	-	timing [1:6]	April		

Relationship with salinity anomalies (phytoplankton)

functional group	category	relation salinity (+,-)	phenology index	month anomaly (salinity)	relation salinity (+,-)	season anomaly (salinity)
coccolithophores	*Emiliana huxleyi*					
coccolithophores	total coccolithophores	-	timing[7:12]	September		
coccolithophores	undetermined coccolithophores					
diatoms	*Chaetoceros affinis*	+	duration	Feb, Mar	+	winter
diatoms	*Chaetoceros anastomosans*					
diatoms	*Chaetoceros simplex*					
diatoms	*Chaetoceros socialis*	-	start 25%	June		
diatoms	*Chaetoceros tenuissimus*	-	start25%	March, April	-	spring
diatoms	*Dactyliosolen phuketensis*					
diatoms	*Minidiscus comicus*	+	timing[1:12]	June	+	spring
diatoms	pennate diatoms >10 µm					
diatoms	*Proboscia alata*					
diatoms	*Pseudo-nitzschia multistriata*					
diatoms	*Skeletonema menzelii*					
diatoms	*Thalassionema nitzschioides*					
diatoms	*Thalassiosira mediterranea*	+	duration	March		
diatoms	*Thalassiosira* spp.		duration		+	summer
diatoms	total diatoms					
dinoflagellates	*Dinobryon faculiferum*	-	start25%	June, July		
dinoflagellates	*Heterocapsa niei*	+	timing[1:12], start25%	March, April	+	spring
dinoflagellates	Naked dinoflagellates >15 µm	+	timing[7:12]	August		
dinoflagellates	total dinoflagellates					
flagellates	*Diplostauron cf.elegans*					
flagellates	*Pachysphaera* spp.					
flagellates	undetermined phytoflagellates >10 µm					

Phenophases (zooplankton)

#	community	functional group	category	change in start 25% (days)	change in start 25% (time period)	change in middle 50% (days)	change in middle 50% (time period)	change in end 75% (days)	change in end 75% (time period)
1	zoo	copepods	Acartia clausi	-30	II	-57	II	-42	II
2	zoo	copepods	*Acartia negligens*						
3	zoo	copepods	Calanidae juveniles						
4	zoo	copepods	Calanoid juveniles						
5	zoo	copepods	Calocalanus spp.						
6	zoo	copepods	*Centropages kroyeri*			-46	I		
7	zoo	copepods	*Centropages ponticus*						
8	zoo	copepods	*Centropages violaceus*			-30	II		
9	zoo	copepods	*Clausocalanus arcuicornis*			-25	II		
10	zoo	copepods	*Clausocalanus furcatus*	+215	II				
11	zoo	copepods	*Clausocalanus lividus*	+41	I				
12	zoo	copepods	*Clausocalanus mastigophorus* (females)						
13	zoo	copepods	*Clausocalanus paululus*						
14	zoo	copepods	*Clausocalanus* spp. (males)						
15	zoo	copepods	*Clytemnestra rostrata*						
16	zoo	copepods	*Corycaeus* spp.						
17	zoo	copepods	*Eucalanidae*	+131	I	+102	I	+173	I
18	zoo	copepods	*Farranula rostrata*						
19	zoo	copepods	*Isias clavipes*	-22	II	-119	I	-156	I
20	zoo	copepods	*Microsetella* spp.			-151	I		
21	zoo	copepods	*Nannocalanus minor*						
22	zoo	copepods	*Oithona longispina*	-122	I				
23	zoo	copepods	*Oithona nana*						
24	zoo	copepods	*Oithona plumifera*	+58	II	+55	II		
25	zoo	copepods	*Oithona setigera* (females)	+191	I				

#	community	functional group	category	change in start 25% (days)	change in start 25% (time period)	change in middle 50% (days)	change in middle 50% (time period)	change in end 75% (days)	change in end 75% (time period)
26	zoo	copepods	*Oithona similis* (females)	-78	I	-77	I	-60	I
27	zoo	copepods	*Oithona* spp. males+juveniles						
28	zoo	copepods	Paracalanus denudatus					-26	I
29	zoo	copepods	*Scaphocalanus* spp.						
30	zoo	copepods	*Scolecithricella* spp.						
31	zoo	copepods	*Temora stylifera*						
32	zoo	meroplankton	Bivalve larvae	+13	II				
33	zoo	meroplankton	Cirripedia larvae						
34	zoo	meroplankton	Isopod Epicaridea larvae						
35	zoo	other holoplankton	Appendicularians						
36	zoo	other holoplankton	Chaetognaths	+65	II				
37	zoo	other holoplankton	Gastropods						
38	zoo	other holoplankton	Hydromedusae						
39	zoo	other holoplankton	Ostracods						
40	zoo	other holoplankton	Pisces larvae + eggs						
41	zoo	other holoplankton	Podon + Pleopis	-107	I	-149	I	-140	I
42	zoo	other holoplankton	salps	+200	II	+232	I	+254	I
43	zoo	total	Total copepods						
44	zoo	total	Total zooplankton						

Duration and timing of central tendency (zooplankton)

functional group	category	change in duration (days)	change in duration (time period)	change in timing (month)	change in timing (time period)
copepods	Acartia clausi				
copepods	*Acartia negligens*		II		
copepods	Calanidae juveniles	-167	II	-1.6	II
copepods	Calanoid juveniles	+114	I		
copepods	Calocalanus spp.	+108	II		
copepods	*Centropages kroyeri*				
copepods	*Centropages ponticus*	+30	I		
copepods	*Centropages violaceus*				
copepods	*Clausocalanus arcuicornis*				
copepods	*Clausocalanus furcatus*				
copepods	*Clausocalanus lividus*				
copepods	*Clausocalanus mastigophorus* (females)			-2.4	I
copepods	*Clausocalanus paululus*			-1.5	II
copepods	*Clausocalanus* spp. (males)			-0.7	I
copepods	*Clytemnestra rostrata*	-207	I		
copepods	*Corycaeus* spp.	-105	I		
copepods	*Eucalanidae*				
copepods	*Farranula rostrata*			-1.2	II
copepods	*Isias clavipes*			-4.1	I
copepods	*Microsetella* spp.				
copepods	*Nannocalanus minor*			-2.0	II
copepods	*Oithona longispina*				
copepods	*Oithona nana*	+66	II		
copepods	*Oithona plumifera*	-24	II		
copepods	*Oithona setigera* (females)			-4.7	II

Duration and timing of central tendency (zooplankton continued)

functional group	category	change in duration (days)	change in duration (time period)	change in timing (month)	change in timing (time period)
copepods	*Oithona similis* (females)			-1.6	I
copepods	*Oithona* spp. males+juveniles			-1.1,-1.8	I, II
copepods	Paracalanus denudatus				
copepods	*Scaphocalanus* spp.	-137	I		
copepods	*Scolecithricella* spp.			+1.0	I
copepods	*Temora stylifera*	-7	II		
meroplankton	Bivalve larvae	-11	II		
meroplankton	Cirripedia larvae				
meroplankton	Isopod Epicaridea larvae				
other holoplankton	Appendicularians			-0.8	I
other holoplankton	Chaetognaths	-66	II		
other holoplankton	Gastropods	+197	II		
other holoplankton	Hydromedusae	-168	II		
other holoplankton	Ostracods			-2.0	II
other holoplankton	Pisces larvae + eggs				
other holoplankton	Podon + Pleopis			-4.1	I
other holoplankton	salps	+235	I	+5.5	I
total	Total copepods			-0.3	I
total	Total zooplankton			-0.3, -0.8	I, II

Relationship with temperature anomalies (zooplankton)

functional group	category	relation temp (+,-)	phenology index	month anomaly (temp)	relation temp anom (+,-)	season anomaly (temp)
copepods	Acartia clausi					
copepods	*Acartia negligens*					
copepods	Calanidae juveniles					
copepods	Calanoid juveniles					
copepods	Calocalanus spp.					
copepods	*Centropages kroyeri*					
copepods	*Centropages ponticus*					
copepods	*Centropages violaceus*					
copepods	*Clausocalanus arcuicornis*					
copepods	*Clausocalanus furcatus*					
copepods	*Clausocalanus lividus*					
copepods	*Clausocalanus mastigophorus* (females)					
copepods	*Clausocalanus paululus*					
copepods	*Clausocalanus* spp. (males)	-	timing [1:6]			spring
copepods	*Clytemnestra rostrata*					
copepods	*Corycaeus* spp.					
copepods	*Eucalanidae*					
copepods	*Farranula rostrata*	+	timing[1:6]			spring
copepods	*Isias clavipes*					
copepods	*Microsetella* spp.					
copepods	*Nannocalanus minor*					
copepods	*Oithona longispina*					
copepods	*Oithona nana*					
copepods	*Oithona plumifera*					
copepods	*Oithona setigera* (females)	-	timing [1:12]			autumn

Relationship with temperature anomalies (zooplankton continued)

functional group	category	relation temp (+,-)	phenology index	month anomaly (temp)	relation temp anom (+,-)	season anomaly (temp)
copepods	*Oithona similis* (females)					
copepods	*Oithona* spp. males+juveniles					
copepods	Paracalanus denudatus					
copepods	*Scaphocalanus* spp.					
copepods	*Scolecithricella* spp.					
copepods	*Temora stylifera*	+	duration			spring
meroplankton	Bivalve larvae	+	start 25%			summer
meroplankton	Cirripedia larvae	-	timing [1:6]			winter
meroplankton	Isopod Epicaridea larvae					
other holoplankton	Appendicularians					
other holoplankton	Chaetognaths					
other holoplankton	Gastropods					
other holoplankton	Hydromedusae					
other holoplankton	Ostracods					
other holoplankton	Pisces larvae + eggs					
other holoplankton	Podon + Pleopis					
other holoplankton	salps	+	timing [1:12]			summer
total	Total copepods	-	timing [1:6]			summer
total	Total zooplankton	-	timing [1:6]			summer

Relationship with chl *a* anomalies (zooplankton)

community	functional group	category	relation chl *a*	phenology index	season anomaly (chl a)
zoo	copepods	Acartia clausi			
zoo	copepods	*Acartia negligens*			
zoo	copepods	Calanidae juveniles			
zoo	copepods	Calanoid juveniles			
zoo	copepods	Calocalanus spp.			
zoo	copepods	*Centropages kroyeri*			
zoo	copepods	*Centropages ponticus*			
zoo	copepods	*Centropages violaceus*			
zoo	copepods	*Clausocalanus arcuicornis*	+	middle50%	winter, spring
zoo	copepods	*Clausocalanus furcatus*			
zoo	copepods	*Clausocalanus lividus*			
zoo	copepods	*Clausocalanus mastigophorus* (females)			
zoo	copepods	*Clausocalanus paululus*			
zoo	copepods	*Clausocalanus* spp. (males)	+	timing [1:6]	spring
zoo	copepods	*Clytemnestra rostrata*			
zoo	copepods	*Corycaeus* spp.			
zoo	copepods	*Eucalanidae*			
zoo	copepods	*Farranula rostrata*	-	timing [1:6]	spring
zoo	copepods	*Isias clavipes*	+	timing[1:12]	spring
zoo	copepods	*Microsetella* spp.			
zoo	copepods	*Nannocalanus minor*	-	timing[7:12]	summer
zoo	copepods	*Oithona longispina*	+	start25%	spring
zoo	copepods	*Oithona nana*	+	start25%	winter
zoo	copepods	*Oithona plumifera*			
zoo	copepods	*Oithona setigera* (females)	-	timing[1:12]	summer, autumn

Relationship with chl *a* anomalies (zooplankton continued)

community	functional group	category	relation chl *a*	phenology index	season anomaly (chl a)
zoo	copepods	*Oithona similis* (females)			
zoo	copepods	*Oithona* spp. males+juveniles			
zoo	copepods	Paracalanus denudatus			
zoo	copepods	*Scaphocalanus* spp.			
zoo	copepods	*Scolecithricella* spp.			
zoo	copepods	*Temora stylifera*			
zoo	meroplankton	Bivalve larvae			
zoo	meroplankton	Cirripedia larvae			
zoo	meroplankton	Isopod Epicaridea larvae			
zoo	other holoplankton	Appendicularians			
zoo	other holoplankton	Chaetognaths			
zoo	other holoplankton	Gastropods			
zoo	other holoplankton	Hydromedusae			
zoo	other holoplankton	Ostracods			
zoo	other holoplankton	Pisces larvae + eggs	-	timing [1:6]	spring
zoo	other holoplankton	Podon + Pleopis			
zoo	other holoplankton	salps	-	timing[1:12], start25%	spring
zoo	total	Total copepods	+	timing [1:6]	summer
zoo	total	Total zooplankton	+	timing [1:6]	summer

ACKNOWLEDGEMENTS

I wish to acknowledge the funding and support to carry out this project from the Open University in London and Stazione Zoologica Anton Dohrn of Napoli, Italy

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I wish to gratefully acknowledge the continued efforts of the Stazione Zoologica to maintain long-term monitoring in the Gulf of Naples in light of reduced funding for scientific research on a global scale. I thank the esteemed Captain and crew of R/V Vettoria, and scientific support that regularly sample station MareChiara. I wish to deeply thank all scientific staff of SZN, LEEP and MECA for their ongoing commitment to the time series.

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I wish to acknowledge my director of studies - Maria Grazia Mazzocchi, and supervisors Adriana Zingone and P. Chris Reid for the opportunity to pursue this project within the scientific context of an international long-term time series, and a historical context within the halls of the first and oldest zoological station in Europe.

I wish to thank members of the Mazzocchi "zoo-lab" for their support, patience and fruitful scientific discussions over the last 3 years. Additional thanks extended to former lab members L.Dubroca, and G. Bianco for assistance with R.

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I wish to extend my deep gratitude for the professional and personal support and guidance that many have maintained throughout the course of three years living abroad. I thank Dr. L. Moore for the continued collaboration and interest in my previous work.

Dedication

I wish to dedicate this work to the late G.F.Collins, civil engineer, meteorologist, scientist, grandfather, gardener, thinker and careful observer of all things wild and beautiful. Thank you for teaching me the skills to watch, listen, draw, record and ask questions – all the best skills in a scientist! God grant me the strength, fortitude and perseverance to write and deliver the message in your name.

27th, September, 2013